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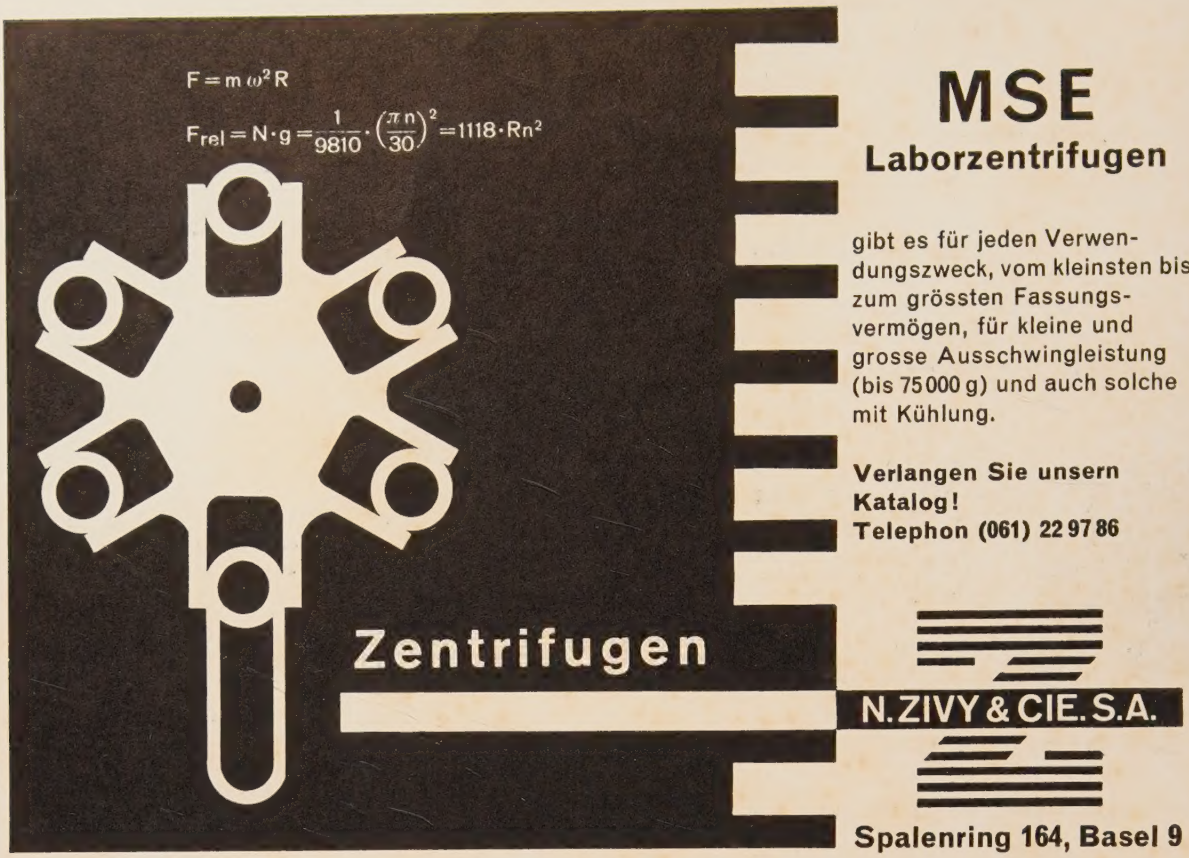

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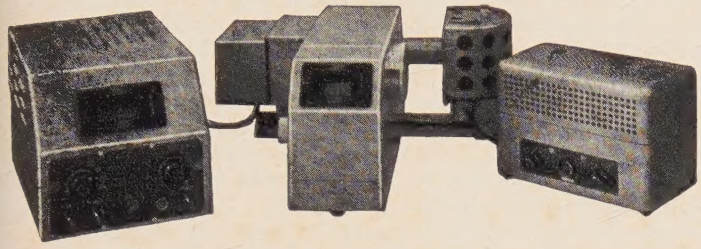

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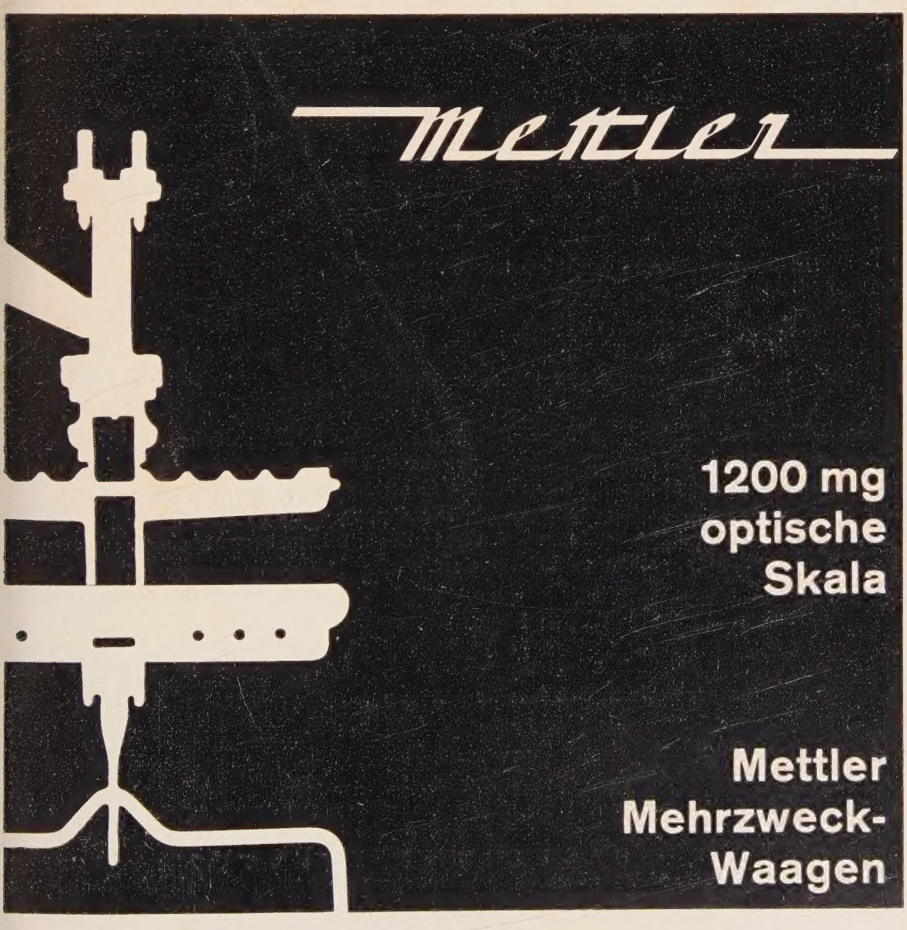

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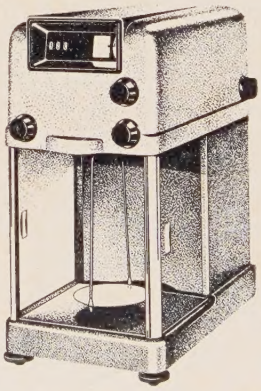

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Induction of Specific Differentiation by Samples of Proteins and Nucleoproteins in the Isolated Ectoderm of *Triturus*-gastrulae

By Tuneo Yamada*

Transplantation and explantation experiments of Holtfreter, Chuang, Toivonen, Rotmann, Fujii, Okada, Hama, Engländer, Kawakami, Vahs and others have established that under the influence of many adult tissues of various vertebrates the ectoderm of amphibian embryos can differentiate in the definite direction characteristic for each adult tissue. Almost all principal organ rudiments belonging to the ectoderm and mesoderm are induced in the presumptive ectoderm of *Triturus*-gastrulae in this way. With few exceptions each adult tissue induces not a single kind but several kinds of structures. However, the array of structures produced under the influence of one of those adult tissues is usually not chaotic but has a distinct regional character. Thus several tissues, including the liver of the guinea pig, induce in the ectoderm archencephalic structures[1], other groups of tissues, including the kidney of the guinea pig, induce spino-caudal and deuterencephalic structures[2], and still other groups of tissues, including the bone marrow of the guinea pig, induce almost exclusively trunk-mesodermal structures[3]. This phenomenon, often referred to as the heterogeneous induction, deserves attention not only of embryologists but of all biologists who are interested in the mechanism of specific differentiation, because it presents a unique possibility of a chemical control of various specific differentiation processes. Although many papers[4] have been devoted to the study of the chemical aspect of the phenomenon, we are still far from understanding the chemical mechanism involved. In the present article, recent progress made in our laboratory in this field will be briefly reviewed.

The method of these experiments consists in fractionation of the extract from some adult tissues with a typical regional inductive effect, and in testing those fractions for their inductive effect on the isolated ectoderm of young gastrulae of the Japanese newt, *Triturus pyrrhogaster*. Chemical and physical characterization of the sample was tried so far as possible. The embryological test was made by implanting a small piece of the sample between two pieces of the isolated ectoderm. The ectodermal pieces fused together to form a vesicle enclosing the implant which was allowed to exert its morphogenetic effect on the internal surface of the ectoderm. The explants were cultured in Holtfreter solution adjusted to pH 7·2–7·4 for 9 to 14 days, exceptionally even for 39 days at 18°C. Differentiation of the explant was studied in microscopic sections. Without an implanted sample, the isolated ectoderm differentiated epidermis cells alone under the experimental conditions adopted.

Regional Inductive Effect of Pentose Nucleoprotein (PNP) from the Liver and Kidney

It had been established by Toivonen[5] that ethanol-treated liver tissue of the guinea pig induces archencephalic structures which can be accompanied by deuterencephalic structures. Working in our laboratory, Hayashi[6] found that 0·14 *M* NaCl extract of the tissue has the same effect, when tested as the ethanol-precipitate. The extract was fractionated by giving streptomycin sulfate. The precipitate obtained was washed repeatedly with a solution of streptomycin sulfate, suspended in a borate buffer and dialyzed. On chemical analysis the sample thus obtained was found to be pentose nucleoprotein (PNP)[7]. The electrophoretic pattern of the sample indicated a single boundary whose mobilities were found to be $-5{\cdot}1 \sim -5{\cdot}4$ ($cm^2/sV \times 10^{-5}$) at the descending limbs and $-6{\cdot}0 \sim -6{\cdot}3$ at the ascending limbs, when determined in 0·2 μ phosphate buffer at pH 7·5 and in 0·2 μ borate buffer at pH 8·5. The ethanol precipitate of the sample induced in the isolated ectoderm archencephalic

* Biologcial Institute, Faculty of Science, Nagoya University.

[1] Eye, fore-brain, nose and lens are designated collectively as archencephalic structures.

[2] Spinal cord, tail-bud, tail-somites and tail-notochord are collectively called spino-caudal structures. Mid-, hind-brain and ear vesicle are called deuterencephalic structures.

[3] Trunk-mesodermal structures include trunk-notochord, trunk-somites, pronephros, blood cells, limb-bud and mesothelium.

[4] S. Toivonen and T. Kuusi, Ann. zool. Soc. 'Vanamo' *13*, 1 (1948). – S. Toivonen, Rev. suisse Zool. *57*, Suppl. 41 (1950). – T. Kuusi, Ann. zool. Soc. 'Vanamo' *14*, 1 (1951); Arch. Biol. *64*, 189 (1953).

[5] S. Toivonen, Ann. Acad. Sci. fenn. [A] *55*, 1 (1940); Exper. *8*, 120 (1952).

[6] Y. Hayashi, Embryologia *3*, 57 (1956).

[7] Y. Hayashi, Embryologia *3*, 57 (1956). – Y. Hayashi and K. Takata (unpublished).

structures in a very high percentage of cases (Fig. 1). On the other hand, the non-precipitable part of the original extract was found to possess weak archencephalic effects. The regional effects of the sample of liver PNP varied according to that of its source material, the archencephalic tendency being combined with the deuterencephalic tendency in varying degree.

A parallel series of experiments was made earlier by YAMADA and TAKATA[8], using as the source material kidney tissue of the guinea pig, which had been shown by TOIVONEN[5] to be a good inducer of spino-caudal and deuterencephalic types. The sample of PNP from the kidney induced spino-caudal and deuterencephalic structures at a high frequency (Fig. 2). No archencephalic structure was recorded in this series. The results fit in with our earlier experiments[9], in which kidney tissue was fractionated into cell components according to the usual technique and these components were tested for their inductive effect. Among the cytoplasmic fractions, the 'small microsome' fraction showed the highest frequency of spino-caudal effect,

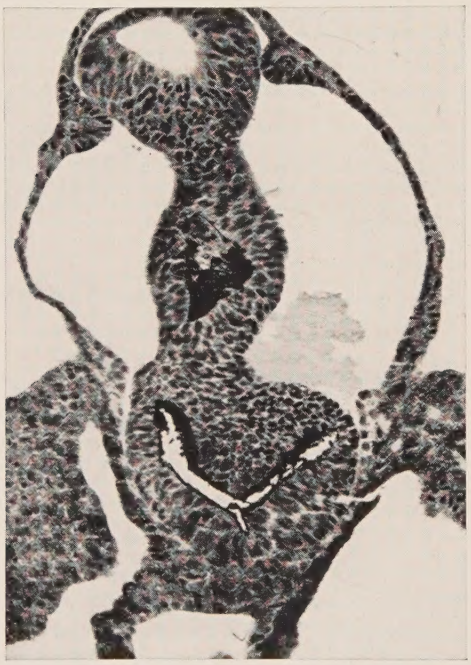


Fig. 1.—Archencephalic induction caused by liver PNP in the isolated ectoderm. Nose, fore-brain and eye-type structure with pigment.

while the 'mitochondria' and 'supernatant' fractions were distinctly weaker in the inductive effect. Very probably our sample of PNP prepared with the streptomycin technique is derived from the microsomes. This was particularly supported by the recent experiments of TAKATA and OSAWA[10] on the appendix of the rabbit, which demonstrated that streptomycin given to an extract of the microsome fraction precipitated the PNP component of the microsomes leaving their non-nucleoprotein component in solution.

Fig. 2.—Effect of a sample of kidney PNP. *a* Somites and a spinal cord-type structure. *b* A deuterencephalon-type structure accompanied by mesenchyme, small ear vesicle-type and ganglion-type structures.

Effect of Liver PNP Samples Purified by Ultracentrifugation

Although the sample of liver PNP obtained with the above method indicated a single boundary on electrophoresis, its sedimentation diagram suggested some heterogeneity. When the sample was fractionated into two or three subfractions by ultracentrifugation, and the PNA content of each subfraction was estimated, the sedimentable subfraction showed a very low value in contrast to a very high value obtained in the non-

Table I. The Pentose Nucleic Acid Content and Inductive Effect of Subfractions of a Pentose Nucleoprotein Sample Separated by Ultracentrifugation

Subfractions	Precipitate of 100,000 × *g*, 1 h	Supernatant 100,000 × *g*, 1 h
PNA Content in μg PNA–P/mg Protein N . .	10·2	92·7
Number of Explants . . .	53	59
Total Neural Induction . .	72%	100%
Archencephalic Induction .	55%	34%
Deuterencephalic Induction	9%	71%
Non-specifiable Induction .	38%	8%

[8] T. YAMADA and K. TAKATA, Embryologia *3*, 69 (1956).

[9] T. YAMADA, K. TAKATA, and S. OSAWA, Zool. Mag., Tokyo *63*, 415 (1954). – T. YAMADA, Symp. Soc. cell. Chem. *4*, 1 (1956).

[10] K. TAKATA and S. OSAWA, Biochim. biophys. Acta *24*, 208 (1957).

sedimentable subfraction (Table I)[11]. This was interpreted to suggest that the original sample of PNP was contaminated with a protein which was sedimentable by the centrifugal force applied. When all those subfractions obtained by ultracentrifugation were studied for their morphogenetic effects on the isolated ectoderm the non-sedimentable subfractions showed a higher frequency of neural and deuterencephalic induction, and a lower frequency of regionally non-specifiable induction, compared with the sedimentable subfractions. The inductive effect of the original PNP sample was found to be closely comparable with that of the non-sedimentable subfraction which seems to represent a very pure sample of PNP, on account of its PNA content.

All these results lead to the conclusion that PNP of the liver itself has a strong inductive effect of archencephalic and deuterencephalic types. It may be added that the PNP sample prepared by the streptomycin technique does not lose its inductive effect after extraction with various organic solvents.

Significance of Nucleic Acid- and Protein-Components for the Induction caused by PNP

The next question raised was the relative role of protein- and nucleic acid-components of PNP in the induction caused by the latter. In an attempt to answer this question ribonuclease, pepsin and trypsin were used to attack one of these components leaving the other more or less intact.

In the first experiment[11] of this group the sample of liver PNP was denatured with ethanol, suspended in a borate buffer of pH 7·6 and incubated with crystalline ribonuclease for 7 h at 28°C under continous dialysis. After the incubation, the sample contained 3·5 μg PNA-P per mg protein N in contrast to 86·6 μg PNA-P of the control sample. The both samples were tested for the regional inductive effect. The very high frequency of the over-all induction of the control sample remained unchanged in the experimental series, and small differences of frequency in regional types observed between the experimental and control series were found to be not significant.

In the second experiments[11] with ribonuclease, liver PNP purified by ultracentrifugation at 100,000 × *g* for 1 h and not denatured with ethanol, was used as the substrate. Incubation with crystalline ribonuclease was made in a saline of pH 7·6 at 22°C for 3·5 h under constant dialysis. At the end of incubation, an equal volume of saturated ammonium sulfate was added to the mixture. The precipitate obtained was used as the sample for the induction experiment after a dialysis and ethanol-precipitation. Whereas the control sample contained a large amount of PNA, the treated sample showed only a trace of the latter (Table II). As indicated in the Table, the high over-all induction frequency of the control sample was unaffected by the treatment. The frequency of archencephalic, deuterencephalic and non-specifiable inductions also remained unchanged after the treatment.

Table II. Inductive Effect of PNP Purified by Ultracentrifugation and Treated with Ribonuclease

	Control	Experimental
PNA Content in μg PNA–P per mg Protein N	148·6	0·84
Number of Explants	49	52
Total Neural Induction	100%	100%
Archencephalic Induction	14%	15%
Deuterencephalic Induction	82%	77%
Non-specifiable Induction	18%	17%

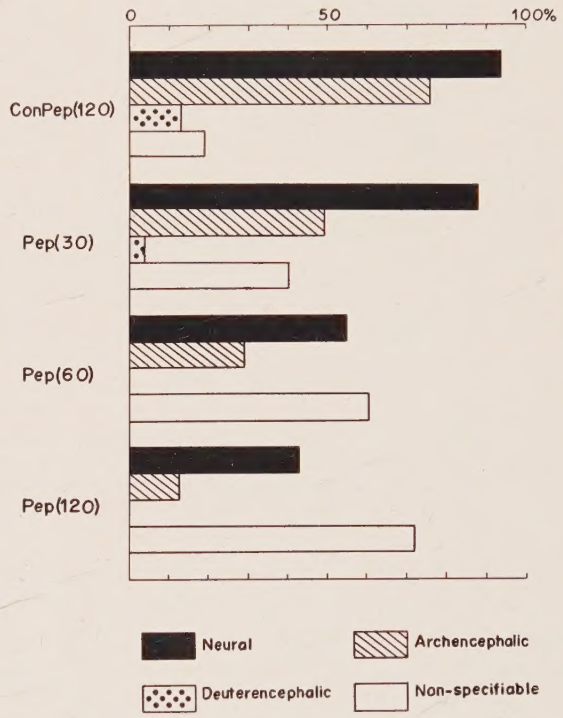


Fig. 3.—Diagrams showing progressive suppression of the inductive effect of a sample of liver PNP treated with pepsin for 0 min (ConPep120), 30 min (Pep30), 60 min (Pep60), and 120 min (Pep120). The inductive frequency of each type of induction is expressed by the percentage of the number of explants containing the type in question against the total number of explants in the series concerned (Hayashi).

In the further series of experiments[11], the effect of crystalline pepsin on the inducing ability of the sample of liver PNP was studied by incubating the sample with the enzyme for 30 min, 60 min, and 120 min at pH 4·0. At the end of the incubation period, the pH was adjusted to 7·5 and ethanol was added to the reaction mixture to precipitate the sample to be tested. As is clear from Figure 3, a progressive decrease of archencephalic and deuterencephalic induction and

[11] Y. Hayashi (unpublished).

concomitant increase in the non-specifiable induction were registered. This can be interpreted to indicate that under the action of the enzyme the formation of essential structures of the central nervous system was suppressed, while the differentiation of non-axial structures derived from the neural fold or of unorganized neural tissue was enhanced.

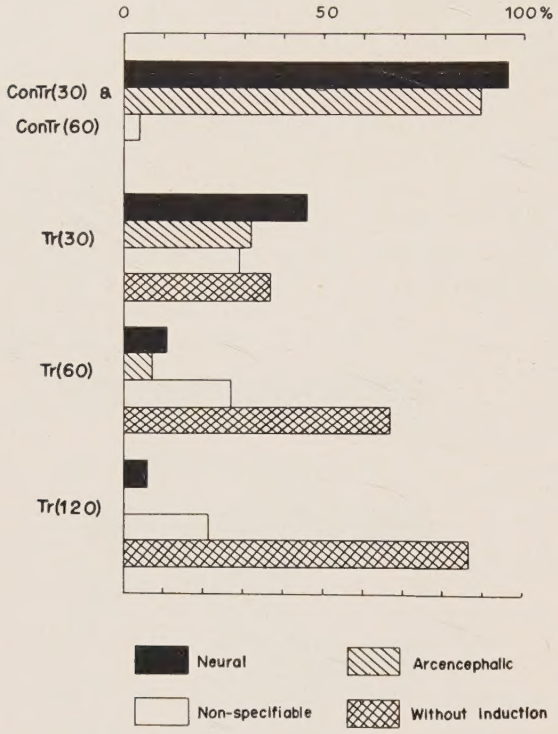


Fig. 4. – Diagrams showing progressive suppression of the inductive effect of a sample of liver PNP by a treatment with trypsin for 10 min (ConTr30, 60), 30 min (Tr30), 60 min (Tr60), and 120 min (Tr120). (HAYASHI).

In a comparable series of experiments[11], the effect of crystalline trypsin was studied after the same time-intervals. After each incubation period, the reaction mixture was heated at 85°C in 95% ethanol in order to inactivate the enzyme. The result demonstrated a progressive suppression of archencephalic induction, leading to a complete disappearance of the latter at the end of 120 min-treatment (Fig. 4). The non-specifiable induction increased weakly up to 60 min, while the number of explants without any induction increased steadily during the whole incubation period to attain the majority of cases at the end of 120 min-treatment. The effect of the heat-treatment was carefully controlled with a special series of experiments, which demonstrated that the treatment does not change the over-all induction frequency but changes the regional effect so that the archencephalic type alone is induced after the treatment.

Taken altogether, the results of the experiments with enzymes harmonize with each other in indicating that in the induction caused by liver PNP the main role is played by the protein-component and not by the nucleic acid-component. This conclusion is compatible with the observations[12] that PNAs from various sources give only weak inductions of the non-specifiable or of archencephalic type in the competent ectoderm. There is also an increasing body of evidence[13] suggesting the inability of ribonuclease to suppress the inducing power of tissues and extracts.

The Chemical Agent Responsible for the Mesodermal Induction by the Bone Marrow

Although in the liver and kidney of the guinea pig, the PNP fraction carries the inductive ability characteristic for each tissue, this is not the case for the bone marrow, the potent inducer of mesodermal structures discovered by TOIVONEN[14]. This was demonstrated in the following two series of experiments[15].

As the starting sample, 0·14 *M* NaCl extract of bone marrow tissue of the guinea pig was precipitated with ethanol and tested for the regional inductive effect as usual. It induced trunk-notochord (50%), trunk-somites (45%), pronephros (45%), blood island (55%) and other mesodermal structures. Neural structures were obtained only very infrequently and in small amounts, always accompanying mesodermal structures.

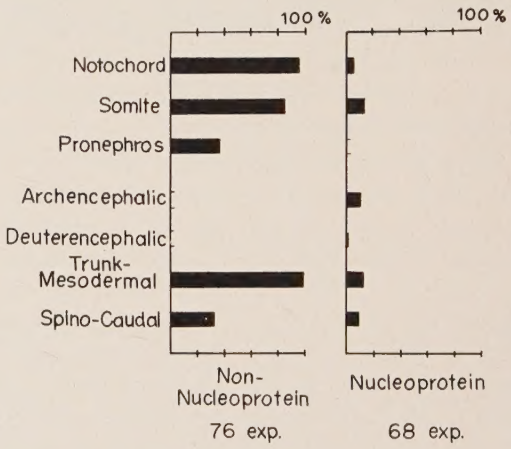


Fig. 5. Diagrams showing the inductive effect of nucleoprotein fraction and non-nucleoprotein fraction from the bone marrow extract.

The inductive effect of the extract was thus closely comparable with that of the original tissue. The ex-

[12] T. KUUSI, Arch. Biol. *64*, 189 (1953). – T. YAMADA, K. TAKATA, and S. OSAWA, Embryologia *2*, 123 (1954). – H. TIEDEMANN and H. TIEDEMANN; Hoppe-Seiler's Z., *306*, 132 (1957).

[13] T. KUUSI, Ann. zool. Soc. 'Vanamo' *14*, 1 (1951); Arch. Biol. *64*, 189 (1953). – H. ENGLÄNDER, A. G. JOHNEN, and W. VAHS, Exper. *9*, 100 (1953). – Y. HAYASHI, Embryologia *2*, 145 (1955). – H. ENGLÄNDER and A. G. JOHNEN, J. Embryol. exp. Morphol. *5*, 1 (1957). – W. VAHS, Roux' Arch. *149*, 339 (1957).

[14] S. TOIVONEN, Arch. Soc. 'Vanamo' *7*, 113 (1953); J. Embryol. exp. Morphol. *1*, 97 (1953); *2*, 239 (1954); Ann. Acad. Sci. fennicae [A IV] *22*, 1 (1954).

[15] T. YAMADA, Y. HAYASHI, K. TAKATA, and S. OSAWA (unpublished).

tract was fractionated into two fractions by the use of streptomycin sulfate. The nucleoprotein fraction which contained the bulk of PNA present in the original extract showed very weak inductive effect of neural and mesodermal nature. A large number of explants failed to show any sign of induction at all (Fig. 5). On the other hand, the non-precipitable fraction which contained only a very small amount of PNA proved to be a potent inducer of trunk-mesodermal structures. The effect of the fraction was characterized by very frequent occurrence and good differentiation of the notochord and somites (Fig. 6). Spino-caudal structures including the spinal cord and tail-bud often accompanied the trunk-mesodermal structures.

In the other series of experiments, the same saline extract was fractionated into four fractions according to the scheme shown in Figure 7. Those fractions were tested for their regional effects by the usual technique. As shown in Figure 8 the microsome fraction and final supernatant fraction were very poor in inductive activity, while both acid-precipitable fractions induced typical trunk-mesodermal structures in all explants. In most cases, the whole mass of the explant was converted into mesodermal structures, except the thin layer of the epidermis covering the surface. Very high frequency of notochord and somites was characteristic for both series. Judged from the mode of separation, both the acid-precipitable fractions may be chemically not much different. An electrophoretic study of the acid-precipitable fraction showed at least two boundaries. The fast moving component which represented only a small fraction of the material showed a mobility comparable with that of PNP of liver and kidney tissues and was assumed to be PNP of the supernatant fraction. The other boundary representing the bulk of the fraction was very slow in movement and assumed to be a protein free of nucleic acid.

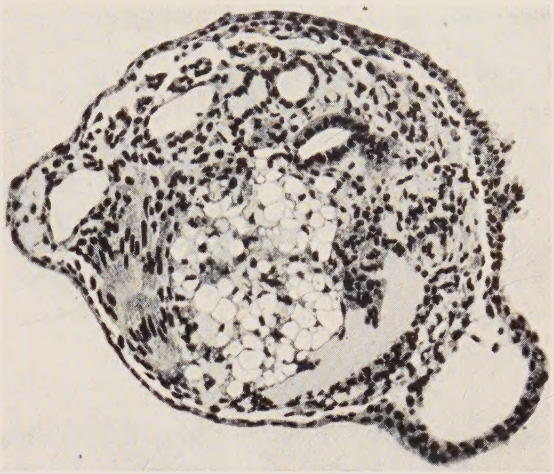


Fig. 6.—Notochord, somites and pronephros induced by the non-nucleoprotein fraction of the bone marrow in the isolated ectoderm.

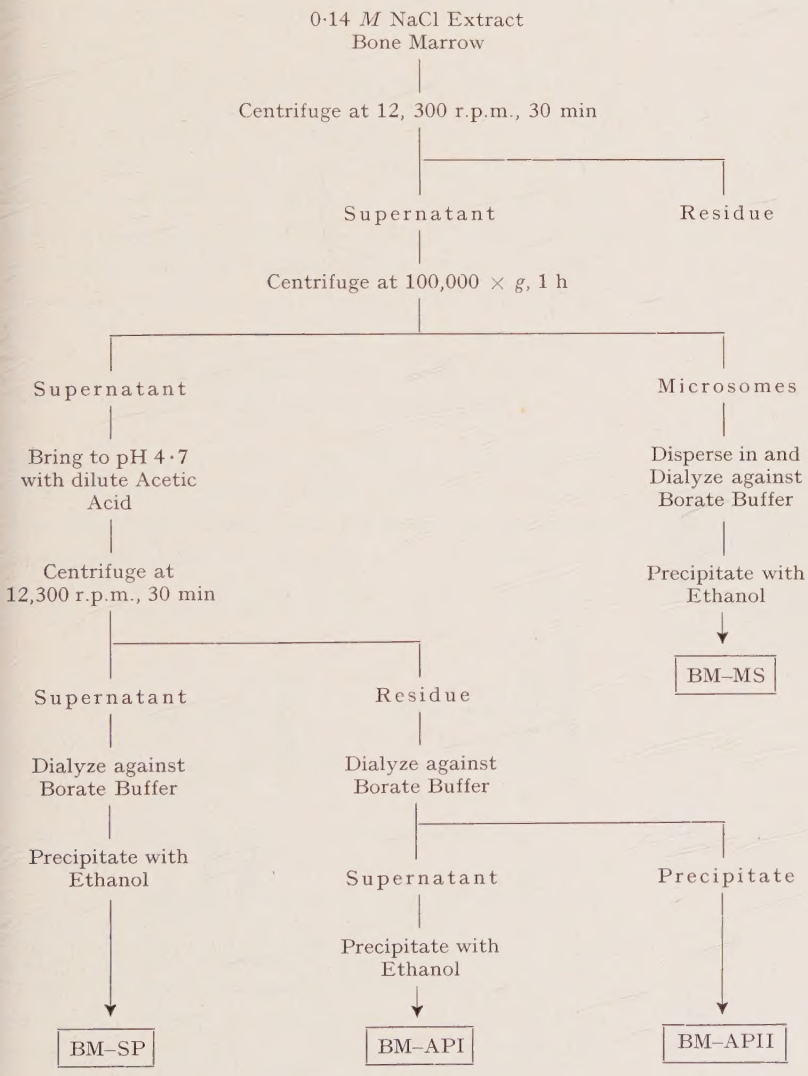


Fig. 7.—The scheme of fractionation of the bone marrow.

Thus intercellular localization of the inductive ability of the bone marrow is quite different from that of the liver and kidney where, as stated, the microsomes appear to be the most important site of localization in the cytoplasm.

The data reviewed above, together with contributions of KUUSI[16] and of TIEDEMANN and TIEDEMANN[17],

[16] T. KUUSI, Ann. zool. Soc. 'Vanamo' *14*, 1 (1951); Arch. Biol. *64*, 189 (1953).

[17] H. TIEDEMANN and H. TIEDEMANN, Hoppe-Seyl. Z. *306*, 7 (1957).

demonstrate that in the differentiated tissue cells a number of proteins or proteids are present which give different regional effects in the same reacting system under identical experimental conditions. They may be called the specific inducing substances. However there

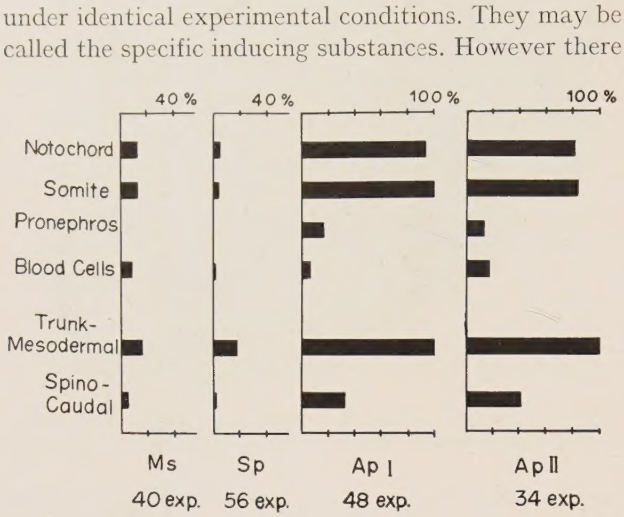


Fig. 8. — Diagrams showing the inductive effect of microsome fraction (Ms), final supernatant (Sp), acid-precipitable fraction of the supernatant I and II (Ap I, Ap II) of the bone marrow (see Fig. 7).

are grounds to assume that their regional specificity is not absolute. For instance, the regional effect of many samples can be shifted or altered by treating them with different physical or chemical agents. Further, by combining one of the samples with another the regional effect can be obtained, which is not produced by either

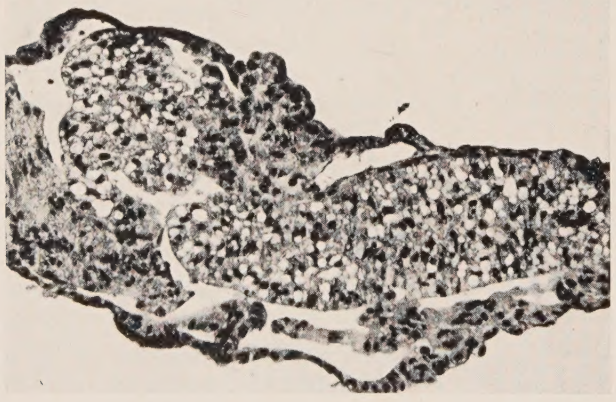


Fig. 9. — Notochord and somites induced by the acid-precipitable fraction II of the bone marrow.

of the two samples[18]. The former type of evidence[19] was first demonstrated in the experiments using the tissue as the inducer, and was later extended to extracts and fractions or even to samples of considerable purity. They are of special interest for embryologists, because they suggest the interrelationship of various regional differentiations and eventually the physico-chemical basis of regionality. The following data indicate that also in the PNP samples alteration of the regional inductive effect does occur: a liver PNP sample with a considerable deuterencephalic tendency induced only archencephalic structures after a short heat-treatment. Further, a kidney PNP sample possessing spino-caudal and deuterencephalic effects in the fresh condition induced archencephalic structures after a longer standing in the solution. Especially suggestive results were obtained by HAYASHI[20] when he studied the effect of pepsin on the sample of kidney PNP. Addition of the enzyme to the sample at a sub-optimum condition resulted in appearance of archencephalic effects and suppression of the spino-caudal tendency. A similar effect of pepsin has been reported by TIEDEMANN and TIEDEMANN[17] on a sample of protein from the extract of the chick embryo. As to the bone marrow and its fractions, there have been no data in favour of a change in regionality. According to TOIVONEN[21], a short heat-treatment of the tissue leads to an almost complete loss of inductive ability. However, for the frog skin, the other inducer of mesodermal structures, OKADA[22] demonstrated the capacity of neural induction after heat-treatment. In the following experiments, a clear shift in regional effects was demonstrated for the bone marrow by a progressive heat-treatment of short duration[23].

Progressive Shift of the Regional Effect by a Heat-Treatment of the Bone Marrow

Small pieces of frozen tissue were spread on a glass plate in a thin layer and exposed to steam in a steam-sterilizer for 25 s, 40 s, 60 s, and 150 s, and then quickly dipped in cold 90% ethanol. The morphogenetic effects of these samples were tested as

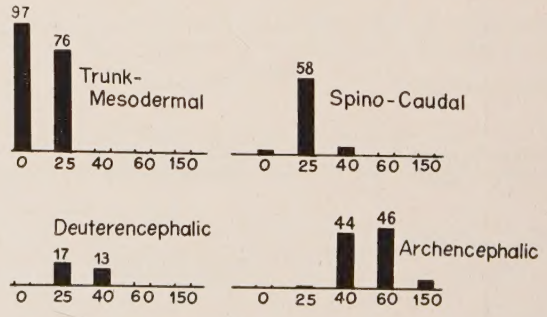


Fig. 10. — Diagrams showing the frequency of various regional types of induction caused by the bone marrow treated with steam for 0 s (0), 25 s (25), 40 s (40), 60 s (60), and 150 s (150).

[18] S. TOIVONEN and E. SAXÉN, Exp. Cell. Res. Suppl. *3*, 346 (1955). – K. YAMANA and I. KAWAKAMI, Zool. Mag., Tokyo *66*, 58 (1957). – T. YAMADA, Zool. Mag., Tokyo *66*, 60 (1957).

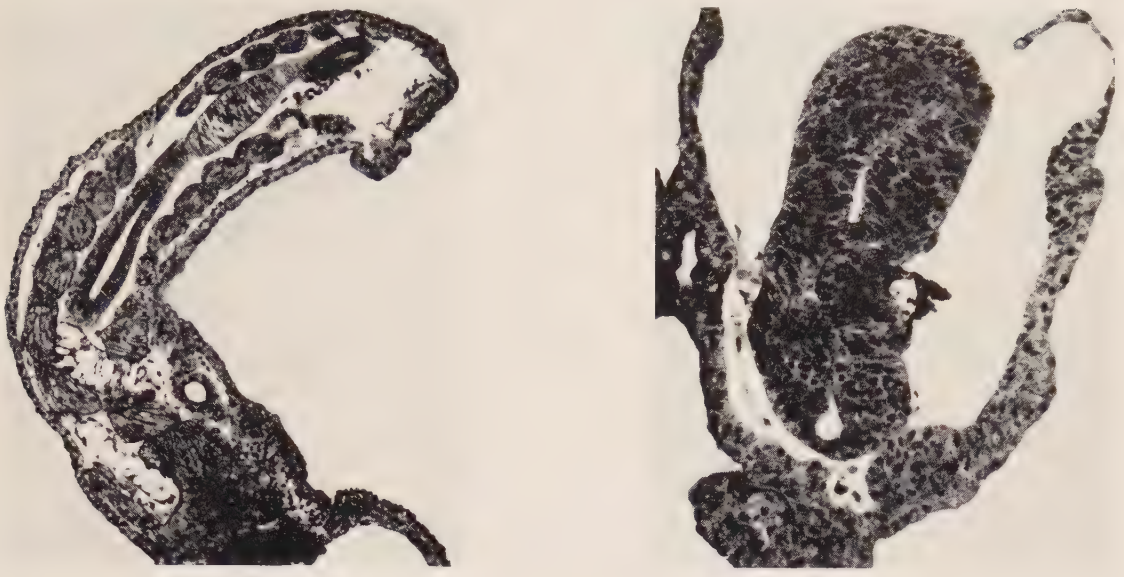
[19] H. H. CHUANG, Roux'Arch. *139*, 556 (1939); *140*, 25 (1940). – H. ENGLÄNDER, A. G. JOHNEN, and W. VAHS, Exper. *9*, 100 (1953). – T. YAMADA and K. TAKATA, J. exp. Zool. *128*, 291 (1955). – W. VAHS, Z. Naturf. *10b*, 412 (1955). – I. KAWAKAMI and S. MIFUNE, Mem. Fac. Sci. Kyushu Univ. [E] *2*, 141 (1957). – H. TIEDEMANN and H. TIEDEMANN, Hoppe-Seyl. Z. *306*, 7 (1957).

[20] Y. HAYASHI (unpublished).

[21] S. TOIVONEN, Ann. Acad. Sci. fennicae [A IV] *22*, 1 (1954).

[22] Y. K. OKADA, Proc. imp. Acad. Japan *24*, 22 (1948).

[23] T. YAMADA, Zool. Mag., Tokyo *66*, 60 (1957).



A *B*

Fig. 11.—(*A*) Typical spino-caudal induction caused by 25 s-steamed bone marrow tissue. Spinal cord, notochord, somites and ear vesicle are visible. (*B*) An archencephalic brain induced by 40 s-steamed bone marrow tissue.

usual and compared with those of the untreated tissue fixed with cold 90% ethanol. Expressed in terms of regional types, a progressive shift in the quality of induction during the whole period was demonstrated (Fig. 10): The trunk-mesodermal type of induction diminished slightly within 25 s and then suddenly disappeared. The spino-caudal type was very infrequent at first, became frequent in 25 s, but perfectly vanished subsequently. The deuterencephalic type was represented only weakly in 25 s and 40 s. It might have a peak between two series. The archencephalic type was completely absent in the control, appeared later to increase remarkably during 40 to 60 s steaming and began to decrease after 150 s steaming. Thus the following sequence of regional types occurred during steaming:

trunk-mesodermal→spino-caudal→deuterencephalic →archencephalic

Inspection of earlier data suggests that this sequence can also be applied to other types of inducer: spino-caudal, deuterencephalic and archencephalic inducers. It must be pointed out, however, that concomitant with this change in regionality a gradual suppression of the total induction frequency may occur. We need further analysis to understand the physical basis of the change in inductive quality. It appears not improbable that the protein molecule responsible for the regional induction undergoes a progressive change in configuration under diverse experimental conditions, and this change is reflected in the progressive change in its regional effect. Such a possibility must have an important implication for the problem of embryonic organization, because the above sequence of regionality happens to coincide with the sequence of distribution of corresponding presumptive areas in the embryo[24].

Zusammenfassung

Ein Präparat von Ribonukleoproteiden der Meerschweinchenleber induziert im isolierten Ektoderm der jungen Gastrula von *Triturus pyrrhogaster* archenzephale und deuterenzephale Strukturen. Die Induktionsfähigkeit des Präparates bleibt unverändert nach der Reinigung durch Ultrazentrifugierung oder nach der Entfernung von Ribonukleinsäure mit Ribonuklease. Anderseits kann die Behandlung des Präparates mit Pepsin und Trypsin die Induktionsfähigkeit progressiv und weitgehend inaktivieren. Mit Präparaten von Ribonukleoproteiden der Meerschweinchenniere werden spino-caudale oder deuterenzephale, aber keine archenzephalen Differenzierungen im Ektoderm ausgelöst. Die Induktion der rumpfmesodermalen Strukturen im Ektoderm durch das Meerschweinchenknochenmark ist mit dem bei pH 4,7 ausfällbaren Anteil der überstehenden Flüssigkeit nach der Ultrazentrifugierung des Extraktes verbunden, und nicht mit der Mikrosomenfraktion, wie das in der Leber und Niere der Fall ist. Die qualitative Veränderung der induktiven Fähigkeiten, die das Knochenmark während der progressiven Hitzebehandlung erfährt, wird analysiert und ihre theoretische Bedeutung kurz diskutiert.

[24] For the further discussion cf. F. E. Lehmann, Rev. Suisse Zool, *57*, 141 (1950). – T. Yamada, Embryologia *1*, 1 (1950). – T. Yamada and K. Takata, J. exp. Zool. *128*, 291 (1955); Exp. Cell Res. Suppl. *3*, 402 (1955). – S. Toivonen and L. Saxén, Ann. Acad. Sci. fenn. [A] *30*, 3 (1955).

Brèves communications - Kurze Mitteilungen
Brevi comunicazioni - Brief Reports

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Microbiological Hydroxylation of Certain Indole Alkaloids

While microbiological transformations have been widely studied in the steroid field, there are only few examples of such transformations of alkaloids.

When apoyohimbine (Ia)[1], β-yohimbine methyl ether (IIa)[2], and 3-epi-apoyohimbine (IIIa)[3] were aerobically incubated (200 γ/ml) in deep culture fermenters with a 48 h growth of *Cunninghamella Blakesleana* Lendner on a pea meal medium for 24–72 h they were transformed into more polar compounds. Details of the fermentations (followed by means of paper chromatography in the B5 system of Bush[5]) and the isolation of the fermentation products will be presented in a forthcoming paper.

Paper chromatography of the apoyohimbine fermentations revealed two transformation products of $R_F = 0{\cdot}60$ (green fluorescence after irradiation with U.V.-light) and $R_F = 0{\cdot}38$ (purple spot after spraying with a butanolic solution of diazotized sulfanilamide followed by alkali[6]) respectively. The main transformation products of IIa and IIIa were also detected with this phenol reagent ($R_F = 0{\cdot}18$ and $0{\cdot}34$ respectively).

The structures Ib, IIb, and IIIb were assigned to the 'phenolic' transformation products of Ia, IIa, and IIIa for the following reasons: The microanalysis in connection with their ready solubility in aqueous alkali, their ability to couple with diazonium salts, and the finding of two active hydrogen atoms (Zerewitinoff) indicated the presence of one phenolic hydroxyl group. A 17-enol in II and III could be excluded since the phenolic properties were retained after catalytical hydrogenation of the 16, 17-double bond. Because of the striking resemblance of the ultraviolet spectra of these compounds [λ_{max} 274 mμ, log ε 3·97; 295–300 mμ (shoulder), log ε 3·65; λ_{min} 248 mμ, log ε 3·61 (0·01n methanolic HCl)] to that of serotonin the hydroxyl group could be located to the 10-position[7].

[1] G. Barger and E. Field, J. chem. Soc. *123*, 1038 (1923)

[2] W. O. Godtfredsen and S. Vangedal, Acta chem. Scand. *11*, 1013 (1957).

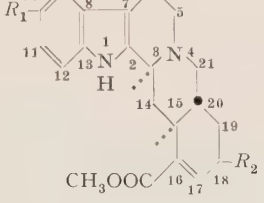
[3] The new compound IIIa was prepared by dehydration of pseudoyohimbine[4] with phosphorus oxychloride in pyridine. M. p. 91–93; $[\alpha]_D^{20} = +85°$ (pyridine).

[4] W. O. Godtfredsen und S. Vangedal, Acta chem. Scand. *10*, 1414 (1956).

[5] W. O. Godtfredsen and S. Vangedal, Acta chem. Scand. *11*, 1013 (1957); *10*, 1414 (1956). – I. E. Bush, Biochem. J. *50*, 370 (1952).

[6] For details, see R. J. Block, E. L. Durrum, and G. Zweig, *Paper Chromatography* (Academic Press, N.Y. 1955), p. 95.

[7] A similar hydroxylation is the recently reported conversion of tryptophan with *Chromobacterium violaceum:* C. Mitoma, H. Weissbach, and S. Udenfriend, Arch. Biochem. Biophys. *63*, 122 (1956).

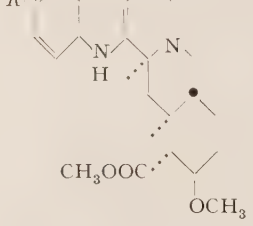


I

a) $R_1 = R_2 = H$

b) $R_1 = OH$, $R_2 = H$ { $C_{21}H_{24}N_2O_3$; m.p. 259–261°C; $[\alpha]_D^{23} = +34{\cdot}5 \pm 1°$ (pyridine)

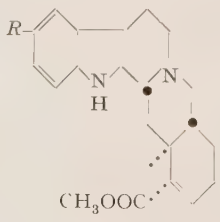
c) $R_1 = H$, $R_2 = OH$ { $C_{21}H_{24}N_2O_3$, HCl; m.p. 273–274°C; $[\alpha]_D^{23} = +64° \pm 1°$ (methanol)



II

a) $R = H$

b) $R = OH$; $C_{22}H_{28}N_2O_4$; m.p. 289–290°C; $[\alpha]_D^{23} = -14 \pm 3°$ (pyridine)



III

a) $R = H$

b) $R = OH$; $C_{21}H_{24}N_2O_3$, $^1/_2\,C_6H_6$; m.p. 146–148°C; $[\alpha]_D^{23} = +40 \pm 1°$ (pyridine)

With vanillin in concentrated hydrochloric acid Ib, IIb, and IIIb gave a very characteristic cornflower blue colour in contrast to the red colour obtained with other alkaloids of the yohimbine type.

The second fermentation product from apoyohimbine was monohydroxylated but not phenolic; its ultraviolet spectrum was practically identical with that of Ia. Although an unambiguous proof of the structure of this compound is still lacking, there is some evidence for the location of the hydroxyl group to the 18-position (Ic): Treatment in chloroform with active manganese dioxide yielded an amorphous product which showed strong infrared absorption bands at 1601, 1680, and 1723 cm^{-1} corresponding to the 16,17-double bond, the keto group and the ester carbonyl in the grouping CH_3OOC- O.

W. O. Godtfredsen, G. Korsby, H. Lorck, and S. Vangedal

Research Department, Leo Pharmaceutical Products Copenhagen, December 3, 1957.

Zusammenfassung

Aerobe Kulturen von *Cunninghamella Blakesleana* Lendner hydroxylieren Apoyohimbin, β-Yohimbinmethyläther und 3-Epiapoyohimbin in der 10-Stellung. Aus Apoyohimbin wurde auch ein zweites Umwandlungsprodukt erhalten, vermutlich 18-Hydroxyapoyohimbin.

A Series of New Compounds Inhibiting the Acetylation of Choline *in vitro*

Introduction.—In previous work, some derivatives of phenylacetic acid were shown to inhibit the enzymatic acetylation of both sulfanilamide[1] and choline[2].

In this paper, we deal with the experiments performed with other acyl-derivatives on the choline acetylating system, in order to establish some relationship between chemical structure and activity, and to investigate the mechanism of the inhibition.

We advanced the hypothesis, as suggested by Cottet for phenylethylacetic acid[3], that these acyl-derivatives might inhibit the synthesis of acetylsulfanilamide and acetylcholine by preventing the acetylation of coenzyme A, the first step, common to both systems, in the acetylating reactions. It was likely that, using chemically preformed acetyl-coenzyme A, the inhibiting activity would disappear.

Experimental.—Choline acetylase was prepared from acetone-dried powder of rabbit brain and partially purified by fractionated ammonium sulfate precipitation, according to Nachmansohn's method[4].

Coenzyme A was supplied from Pabst Laboratories.

Acetyl-Coenzyme A was prepared by acetylating CoA with acetic anhydride. After the acetylation, the preparation was lyophilized to remove acetic anhydride and then dissolved in phosphate buffer at pH 7·2. Chromatographic controls were carried out on an aliquot of the preparation[5].

Inhibiting compounds marked Th have been kindly supplied by Cottet (Theraplix, Paris); compounds marked M.G. were synthetized by Cavallini and Massarani (Laboratori Maggioni, Milano)[6]; compounds marked L were synthetized by Carani (Laboratori Lofarma, Milano).

Acetylcholine assay was performed on the contraction of frog rectus muscle[7]. Similar results were obtained with chemical determinations[8], but we used bioassay as a routine test, since some compounds were found to interfere in the colorimetric reaction. None of the compounds tested, at the doses employed, interfered with the biological assay.

Results

(a) *Inhibition in acetylcholine synthesis with various compounds.* In Table II the inhibiting values obtained with the various substances are presented.

(b) *Experiments with different doses of CoA or choline acetylase.* Some experiments were performed by varying the different components of the enzymatic system.

Increased amounts of choline, acetate or ATP do not affect the inhibiting activity of the tested compounds.

On the contrary, a decrease in the percentage inhibition was obtained by increasing coenzyme A or choline acetylase in the system. Some results are summarized in Table I. The most striking effects were obtained by contemporary increase of CoA and choline acetylase; even when larger amounts of acetylcholine were synthetized, the inhibition disappeared.

Table I

Inhibitor and doses	Enzymatic solution (choline acetylase) in ml	% inhibition	
		in presence of 10 γ of CoA	in presence of 100 γ of CoA
—	0·25	(110) * —	(150) * —
L 10 1 μmole	0·25	77	49
MG 1763 1 μmole	0·25	34	4
—	0·50	(170) —	(212) —
L 10 1 μmole	0·50	49	0
MG 1763 1 μmole	0·50	15	0
—	1	(162) —	(216) —
L 10 1 μmole	1	22	0
MG 1763 1 μmole	1	9	0

* The γ of synthetized acetylcholine are reported in brackets.

(c) *Experiments with acetyl-coenzyme A.*—In Table III are reported the results obtained when chemically preformed acetyl-coenzyme A was substituted to coenzyme A, acetate and ATP. Even at a dose of inhibitor

[1] S. Garattini, C. Morpurgo, and N. Passerini, G. ital. Chemioterapia *2*, 60 (1955); Boll. Soc. ital. Biol. sper. *31*, 1653 (1955).

[2] S. Garattini, C. Morpurgo, B. Murelli, R. Paoletti, and N. Passerini, Arch. int. Pharmacodyn. *109*, 400 (1957).

[3] J. Cottet, A. Mathivat, and J. Redel, Pr. méd. *62*, 939 (1954).

[4] D. Nachmansohn and I. B. Wilson, Advanc. Enzymol. *12*, 259 (1951).

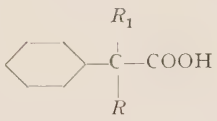
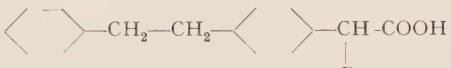
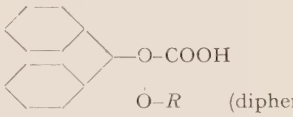
[5] G. D. Novelli, *Methods of Biochemical Analysis*, Interscience Publ. New York *2*, 208 (1955).

[6] G. Cavallini and E. Massarani, Il Farmaco, Ed. scient. *11*, 167 (1956). – G. Cavallini, E. Massarani, D. Nardi, and R. D. Ambrosio, J. Amer. chem. Soc. *79*, 3514 (1957).

[7] H. C. Chang and J. H. Gaddum, J. Physiol. *79*, 225 (1933).

[8] S. Hestrin, J. biol. Chem. *180*, 249 (1949).

Table II

Code number	Formula	% inhibition of acetylcholine synthesis with doses of 0·5 μ moles	1 μ moles	2 μ moles	5 μ moles
					
	R = H R_1 = H (phenylacetic acid)	—	—	0	8
	R = ethyl R_1 = H	—	—	7	17
Th 4141	R = ethyl R_1 = ethyl	—	—	22	27
Th 4142	R = heptyl R_1 = H	27	46	67	100
	R = phenyl R_1 = H (diphenylacetic acid)	20	27	50	74
MG 1271	R = H (diphenylylacetic acid)	20	22	34	80
MG 1753	R = methyl	19	24	54	85
MG 1559	R = ethyl	20	33	52	88
MG 1681	R = propyl	—	39	65	—
MG 1763	R = butyl	25	52	78	100
MG 1765	R = amyl	31	64	90	100
	 (diphenylethanacetic derivatives)				
MG 1713	R = ethyl	20	34	60	90
MG 1777	R = propyl	18	46	78	100
	 (diphenylglycolic derivatives)				
L. 1	R = methyl	—	—	28	48
L. 3	R = propyl	—	—	45	68
L. 4	R = isopropyl	—	—	42	62
L. 10	R = heptyl	51	82	100	—
L. 13	R = propylphenyl	33	75	—	—
L. 14	R = ethyloxyphenyl	55	80	92	100

The enzymatic system was set up as follows: choline chloride 10 μmoles; sodium acetate 10 μmoles; ATP 6 μmoles; cysteine HCl 30 μmoles; KCl 40 μmoles; $MgCl_2$ 0·7 μmoles; $CaCl_2$ 2 μmoles; Na_2HPO_4 1·5 μmoles; DFP 0·1% one drop; choline acetylase 0·25 ml; CoA 10 γ; H_2O to 1 ml; pH 7–7·2. 3 h incubation at 37° C (90–110 γ of acetylcholine were synthesized in the absence of inhibitors).

which inhibits completely choline acetylation also in presence of large amounts of coenzyme A, there is no significant inhibition.

Table III

Inhibitor dose	CoA	AcCoA	Acetylcholine synthesized in γ	% inhibition
—	2 μmoles	–	140	–
L 10 10 μmoles	2 μmoles	–	0	100
—	–	2 μmoles	70	–
L 10 10 μmoles	–	2 μmoles	60	14
—	–	4 μmoles	110	–
L 10 10 μmoles	–	4 μmoles	110	0

The system with AcCoA contained choline 10 μmoles; DFP 0·1% one drop. Choline acetylase 1 ml in both systems.

Discussion.—Many acyl-aromatic acid derivatives have been shown to inhibit acetylcholine synthesis in CoA catalyzed systems with choline acetylase from rabbit brain.

Some speculations about the relationship between chemical structure and inhibiting activity are possible: phenylacetic acid, the first compound studied[9] has only a weak inhibiting activity. The introduction of another aromatic nucleus strongly increases the activity (see diphenylacetic, diphenylglycolic, diphenylylacetic and diphenylethanacetic acids). In every series, the introduction of a side chain steadily increases the activity and such increase is related to the length of the side chain.

As to the inhibiting mechanism, the fact that no inhibition appears when acetyl-coenzyme A is used or

[9] S. K. Korey, D. Nachmansohn, and B. Braganza, J. biol. Chem. *189*, 705 (1951).

when the amounts of coenzyme A and choline acetylase (therefore CoA acetylating system) are increased, suggests that these compounds do not affect the enzymatic transfer of acetic group from acetyl-coenzyme A to choline, but they inhibit the acetylation of coenzyme A. This, moreover, was likely, because our substances inhibit sulfanilamide acetylation also in pigeon liver extracts [2, 10].

Acknowledgement. We wish to thank Dr. E. Mussini for his cooperation in carrying out chromatographic controls on acetyl-coenzyme A.

S. Garattini, C. Morpurgo, and N. Passerini

Institute of Pharmacology, University of Milan (Italy), November 14, 1957.

Riassunto

Numerosi derivati di acidi acil-aromatici si dimostrano attivi nell'inibire la acetilazione della colina catalizzata dal Coenzima A.

Si ritiene che questa inibizione si realizzi attraverso una inibizione della acetilazione del Coenzima A.

[10] S. Garattini, C. Morpurgo, and N. Passerini (in press).

Serological Properties of Poly-L-Tyrosine Derivatives[1]

Sela *et al.*[2] have reported that guinea pigs are sensitized by the injection of polytyrosyl-gelatin, but not by gelatin alone, nor by a copolymer of tyrosine and aspartic acid. Since gelatin has been found to be antigenic in some species[3], it is not clear whether the antigenicity of polytyrosyl-gelatin is due to its gelatin moiety or to its polytyrosyl residue. We have investigated, therefore, the serological behaviour of two polytyrosine derivatives. One of them, poly-L-tyrosine-azophenylarsonate (PTA) was prepared by coupling polytyrosine ($n = 45$)[4] with an excess of diazotized arsanilic acid. The other, poly-L-tyrosyl-gelatin-azophenylarsonate (PTGA) was obtained in the same manner from poly-L-tyrosyl-gelatin[2]. Rabbits were injected subcutaneously with four 30 mg doses of PTA or PTGA directly or after addition of alum and neutralization with alkali. The first three injections were given in intervals of 3 days, the last injection after a further week. One week later the animals were bled and their sera tested with PTA, PTGA and also with arsanil-azo-bovine-γ-globulin (AsBGG) prepared from one gram of bovine γ-globulin (Armour) with 0·1 g of diazotized arsanilic acid. Neither PTA nor PTGA gave any precipitates. However, the serum from a rabbit injected with alum-PTGA gave a distinct precipitin test with AsBGG. When 4·5 ml of the serum were incubated with 0·5 mg AsBGG, a precipitate was obtained which was not noticeably soluble on addition of 0·5 ml of a 2% solution of AsBGG. The insoluble residue, after washing, weighed 2·2 mg; colorimetric comparison of its solution in 1% NaOH with a standard solution of AsBGG showed that it contained 0·2–0·3 mg AsBGG. When three 2 ml samples of the same immune serum were incubated with 0·2 mg PTA, PTGA or AsBGG, only the last substance gave a precipitate; it contained approximately 0·1 mg AsBGG.

We conclude from these results that poly-L-tyrosine-azophenylarsonate is not an antigen, but that poly-L-tyrosyl-gelatin-azophenylarsonate, if injected with alum as adjuvant, induces formation of precipitins which combine with the tyrosine-bound azophenylarsonate groups. Evidently, the nonantigenic poly-tyrosyl-azophenylarsonate acquires antigenic properties by its combination with gelatin.

M. Sela* and F. Haurowitz

Department of Chemistry, Indiana University, Bloomington, Indiana, November 2, 1957.

Zusammenfassung

Polytyrosin und Polytyrosylgelatine wurden mit diazotierter Arsanilsäure gekoppelt und in Lösung oder nach Fällung mit Alaun und Alkali Kaninchen injiziert. Die injizierten Substanzen wurden von keinem der Sera präzipitiert. Hingegen präzipitierte das Serum der mit gefällter Arsanilazo-polytyrosyl-gelatine injizierten Tiere Arsanilazo-Rinderserumglobulin. Wir schliessen daraus, dass Arsanilazopolytyrosin nicht als Antigen wirkt, dass es aber durch Bindung an Gelatine antigene Eigenschaften gewinnt.

* On leave of absence from the Weizmann Institute of Science, Rehovoth (Israel).

[1] This work was supported by research grants of the National Science Foundation and the U.S. Public Health Service, and by contracts of Indiana University with the Office of Naval Research and the Atomic Energy Commission.

[2] M. Sela, E. Katchalski, and A. L. Olitzki, Science *123*, 1129 (1956).

[3] P. Maurer, Arch. Biochem. Biophys. *58*, 205 (1955).

[4] E. Katchalski and M. Sela, J. Amer. chem. Soc. *75*, 5284 (1953).

Sucrose and Starch Synthesis in Sugar Cane Plant

During the investigations on the formation of sucrose in the sugar cane plant it was observed that all parts of the plant except the top node of the matured sugar cane contained only sucrose, glucose and fructose but not starch, at all stages of the development, whereas the top node of the matured sugar cane contained starch. This led us to think that a sucrose-synthesizing enzyme system may be predominant in all parts except the top node of the mature sugar-cane in which the presence of starch-synthesizing enzyme may be a special feature. Hence studies were undertaken to detect the sucrose- and the starch-synthesizing enzyme systems in various tissues of the plant at different stages of development and the results are recorded in this communication.

Young, middle-aged and matured sugar-cane plants were taken for the experiment. Cell-free extracts of leaves, roots, nodes and internodes were prepared as previously described[1] and tested for the presence of sucrose-synthesizing and starch-synthesizing enzyme systems.

For estimation of sucrose-synthesising enzyme system, 2 ml of the assay system contained citrate buffer (pH 6·5), 50 μM; fructose, 60 μM; glucose-1-phosphate,

[1] K. P. Pandya and C. V. Ramakrishnan, Naturwissenschaften *15*, 352 (1956).

	Young sugar cane Specific activity of		Two months old sugar cane Specific activity of		Matured sugar cane	
	Sucrose synthesizing enzyme	Starch synthesizing enzyme	Sucrose synthesizing enzyme	Starch synthesizing enzyme	Sucrose synthesizing enzyme	Starch synthesizing enzyme
Leaves	0·30	—	0·50	—	0·35	—
Roots	0·02	—	—	—	—	—
Nodes	0·15	—	0·20	—	0·10	—
Internodes	0·10	—	0·14	—	0·05	—
Top nodes*	0·10	—	0·20	—	0·01	0·009

* In case of the top node, the sucrose content per mg dry weight of the tissue of the matured sugar cane was only 10% of that of the young sugar cane.

20 μM; enzyme preparation 1 ml (enzyme extracts are prepared in such a way as to contain 20 mg protein per ml in the case of leaf extract whereas about 40 mg of protein in the case of other parts of the plants) and water added to 2 ml. Additions were made in the test tubes and corked well. After addition of the enzyme, the test tubes were incubated for 1 h at 37°C. Enzyme and substrate blanks accompanied the samples. At the end of the incubation period, the contents were centrifuged. Fructose, sucrose and the inorganic phosphate were determined on the supernatant. Fructose was estimated according to Hanes' method[2], the inorganic phosphate according to Fiske and Subbarow[3] and total sucrose according to Roe[4].

For estimation of starch-synthesizing enzyme, the method of Hanes modified by Murthy, Swaminathan and Subramanyan[5] was used. Tests carried out on the extracts showed that they did not possess phosphatase and amylase activities.

Protein was determined by estimating mikro-kjeldahl nitrogen.

For sucrose-synthesizing enzyme, a unit of enzyme activity is defined as that causing a synthesis of μM of sucrose in 1 h and the specific activity as units per mg protein.

For starch-synthesizing enzyme, a unit of enzyme activity is defined as that causing a synthesis of 1 mg of starch in 1 h and the specific activity as units per mg protein.

The results are given in the Table.

From the table, it can be seen that in two months old sugar cane the activity of sucrose synthesizing enzyme system is high and it is less in matured sugar cane. Starch-synthesizing system could not be detected in any other part of the plant except the top node in matured sugar cane where alone the starch was detected. This shows the possibility that, in top node of the matured sugar cane, starch may be synthesized.

The authors thanks are due to Dr. Balakrishna Patel and Ranchodbai Patel of Institute of Agriculture, Anand, for the generous supply of sugar cane plant.

C. V. Ramakrishnan

Biochemistry Department, Faculty of Science, M. S. University of Baroda (India), November 7, 1957.

[2] C. S. Hanes, Biochem. J. *23*, 99 (1929).
[3] C. H. Fiske and Y. Subbarow, J. biol. Chem. *66*, 375 (1925).
[4] J. H. Roe, J. biol. Chem. *107*, 15 (1934).
[5] H. B. N. Murthy, M. Swaminathan and V. Subramanyan, J. sci. industr. Res. *13*, 223 (1954).

Zusammenfassung

Im Gipfelknoten des ausgereiften Zuckerrohrs liess sich die Anwesenheit eines stärkebildenden Enzyms nachweisen, während die anderen stärkefreien Teile dieser Pflanze ein sukrosebildendes Enzymsystem enthalten. Diese Befunde stehen im Einklang mit der Speicherung von Stärke im Gipfelknoten und dem Fehlen von Stärke in den übrigen sukrosehaltigen Pflanzenteilen des Zuckerrohrs.

The Gonad Stimulating Potency of Date Palm Pollen Grains

Several investigators were able to extract estrogenic materials from palm kernels and date palm pollen grains (Butenandt and Jacobi[1], Hassan and Wafa[2] and El-Ridi and Wafa[3]). Recently a gonad-stimulating principle was extracted from pollens. When injected into immature male and female rats, it increased the weights of their gonads and activated them (Soliman and Soliman[4]). Preliminary experiments also indicated that this effect is obtained if defattened pollen grains are fed to immature animals.

In the present investigation, it was planned to determine quantitatively the follicle-stimulating and lutenizing potency of date palm pollen grains (*Phoenix dactylifera* L.). 100 g of pollen grains were freshly obtained. They were defatted with three changes of 200 ml of petrolium ether, then washed with acetone and left to dry at room temperature. The gonadotrophic principles were then extracted, using the method of McShan and Meyer[5]. The extract was then dissolved in 10 ml of distilled water and stored at the temperature of 5°C.

For determination of lutenizing activity, the method adopted by Soliman[6] was used. The plant hormone was compared with a standard preparation of HCG (Physex, Leo). The slope-ratio method of Finney *et al.*[7], was used to evaluate the relative activity. Thirty immature female mice were injected subcutaneously with 20 I.U. of PMS (Antex, Leo). Four days later when the graafian

[1] A. Butenandt and H. Jacobi, Hoppe-Seyl. Z. *218*, 104 (1933).
[2] A. Hassan and H. Abou El Wafa, Nature *159*, 409 (1947).
[3] M. S. El-Ridi and M. Abou El Wafa, Roy. Egypt. Med. Assoc. *30*, 124 (1947).
[4] F. A. Soliman and L. Soliman, Exper., in press (1957).
[5] W. H. Mc Shan and R. K. Meyer, J. biol. Chem. *135*, 473 (1939).
[6] F. A. Soliman, Egypt. Med. Assoc., in press (1957).
[7] D. J. Finney, J. H. Burns, and L. G. Goodwin, *Biological standardization*, 2nd edition (Oxford Univ. Press 1957).

Table I.–LH Content of Date Palm Pollen Grains

Treatment	Body Weight	Average No. of Corpora Hemorrhagica
20 I. U. PMS . . .	8·80	0·40±0·11
1 I. U. HCG . . .	9·80	0·60±0·03
10 I. U. HCG . . .	8·50	4·00±0·35
100 I. U. HCG . . .	9·00	7·60±0·06
1 g Pollen	11·50	1·33±0·09
2 g Pollen	8·80	1·67±0·60

Each group contained 5 animals. ± Standard error.
* All mice given HCG were previously treated with 20 I. U. PMS.
$b \equiv 3{\cdot}50$.

follicles become mature, the mice were divided into six groups of five animals each. Three groups were injected with 1, 10, and 100 I.U. of HCG. One group was injected with extract from 1 g pollen, another with extract from 2 g pollen. The last group was left without any further treatment. Two days later the animals were killed with ether and the number of corpora hemorrhagica formed were used as an index of LH content.

The follicle-stimulating activity of pollens was compared with a standard curve established under the same conditions. The LH augmentation method of Steelman and Pohly[8] was used. Twenty-eight immature female rats were divided into 7 groups and injected with graded doses of PMS to which 20 I.U. of HCG were added. A group of rats was injected with extract from 2 g pollen, and another group with extract from 2 g pollen plus 20 I.U. of HCG. The animals were killed with ether on the fourth day and their body weights and ovary weights were recorded. The data were analyzed statistically.

Table II.–FSH Content of Date Palm Pollen Grains

Treatment	Body Weight	Ovary Weight in mg/100 g Body Weight
Control.	30·25	24·90±1·43
20 I. U. HCG. . .	27·00	41·62±0·50
1 I. U. PMS*. . .	24·67	44·62±0·03
10 I. U. PMS . . .	27·67	241·90±4·98
100 I. U. PMS*. . .	25·00	412·03±7·80
2 g Pollen	29·25	45·15±1·76
2 g Pollen*	32·00	60·22±3·10

Each group contained 4 rats. ± Standard error.
* Twenty I. U. were added to the injected material.
$b \equiv 218$.

When compared with the respective standard curves and by using the formula $\overline{Y}_x = \overline{Y} - b\,(X - \overline{X})$, where $\overline{Y}_x$ represents the average value of response, $\overline{Y}$ the total average response values, b the measure of slope and $\overline{X}$ the total average of the logarithm of the doses used. It was found that 1 g pollen contains 1·05 and 2 g contained 2·06 I.U. of LH activity. The FSH activity was calculated by the same method. It was found that 2 g pollen contained about 1·61 I.U. of PMS. A look at the ovarian weights of rats treated with 2 g pollen (Table II) shows that it is comparable with that of rats treated with 20 I.U. of HCG alone. This shows that the two fractions of LH and FSH are present in a ratio which resulted in the augmentation of their effects. The combined activity of the two fractions of hormones present in 1 g pollen is close from 10 I.U. of HCG.

F. A. Soliman and Amira Soliman

Physiology Department, Faculty of Veterinary Medicine, Cairo University, Giza (Egypt) and Veterinary Research Laboratory, Ministry of Agriculture, Doki (Egypt), Octobre 16, 1957.

Résumé

La principale substance gonadotrophique des grains de pollen des palmiers-dattiers est extraite avec de l'acétone. On a constaté qu'un g de pollen contient 1,05 I.U. de LH et 0,80 I.U. de FSH. L'activité combinée des deux fractions augmente leur effet qui atteint jusqu'à 10 I.U. HCG par g.

[8] S. L. Steelman and F. M. Pohly, Endocrinology *53*, 604 (1953).

DISPUTANDA

Der respiratorische Quotient von Tabakblättern

Der Atmungsquotient (RQ) von grünen Tabakblättern ist bis heute schon von verschiedenen Autoren untersucht worden. So hat Eichenberger[1] gefunden, dass die Blätter der schweizerischen Tabaksorte Mt. Calme brun während des Tages einen RQ aufweisen, der zwischen 1,15 und 1,35 liegt. Frey-Wyssling und Waltz[2] stellten daraufhin eine umfassende Studie über dieses Problem an und untersuchten alle in der Schweiz angebauten Tabaksorten. Sie fanden dabei zum Beispiel für die Sorte Paesana einen RQ von 1,26. Da der Tabak eine Stärkepflanze ist[3], sind vor allem Kohlehydrate als Atmungssubstrate zu erwarten. Das würde einen RQ von ungefähr 1 bedingen. Die beiden Tatsachen, dass das Tabakblatt reich an organischen Säuren ist[3] und dass der RQ auch während der Nacht nie unter 1 sinkt, bewogen Frey-Wyssling und Waltz, eine vom Sukkulententypus[4] verschiedene Atmung für den Tabak zu postulieren. Waltz[5] versuchte darauf, eine Korrelation dieses neuen Atmungstypus mit dem Nitratstoffwechsel erwachsener Pflanzen nachzuweisen. Es gelang ihm dies aber nicht. Auf der andern Seite hatte Roberts[6] schon 1941 den Tabak-RQ untersucht. Er fand ebenfalls hohe Werte bei *Luftfüllung* des Gasraumes der Warburggefässe; bei *Sauerstoffüllung* hingegen erhielt er einen RQ von 1,01. Wir untersuchten die Sorte Paesana, bei der Waltz[5] nach sechsstündigem Aufenthalt im Dunkeln noch einen RQ von etwa 1,27 fand. Die Messungen wurden am Blatt Nr. 18 (vom untersten sichtbaren Blatt an gezählt) von Freilandpflanzen ausgeführt. Um eine Kohlendioxyd-Dunkelfixierung[7], die die Messung eventuell hätte stören können, abklingen zu lassen, wurden die Blätter nach dem Pflücken vorerst 6 h im Dunkeln gelassen. Nachher gingen wir nach der Methode von Waltz[5] vor.

[1] E. Eichenberger, Ber. schweiz. bot. Ges. *62*, 123 (1952).
[2] A. Frey-Wyssling und P. Waltz, Exper. *11*, 178 (1955).
[3] A. I. Smirnow, *Biochemie des Tabaks* (Den Haag 1940).
[4] T. A. Bennet-Clark, Sci. Proc. R. Dublin Soc. *20*, 293 (1932).
[5] P. Waltz, Ber. schweiz. bot. Ges. *67*, 55 (1957).
[6] E. A. H. Roberts, Biochem. J. *35*, 1289 (1941).
[7] R. E. Stutz und R. H. Burris, Plant Physiol. *26*, 226 (1951).

Wir erhielten so die folgenden Werte:

Atmosphäre	Verbrauchter O_2 in mm^3 pro 200 mg Tabakblatt-Frischgewicht und 15 min	Produziertes CO_2 in mm^3 pro 200 mg Tabakblatt-Frischgewicht und 15 min	RQ
Luft	43,2	52,6	1,22
Sauerstoff . .	51,2	51,2	1,00
		(Mittlerer Fehler der Resultate: 5%)	

Diese Messungen zeigen, dass der hohe RQ nur auftritt, weil der Sauerstoff nicht genügend in das Innere des Gewebes diffundieren kann, um einen Sauerstoff-Partialdruck aufrechtzuerhalten, der die aerobe Oxydation der Kohlehydrate erlaubt. Über die Muttersubstanz des Kohlendioxydes der ungenügend mit Sauerstoff versorgten Zellen steht die Diskussion noch offen.

Aus den oben erhaltenen Daten berechneten wir die Grenzschnittdicke d, die nötig ist, um die Gewebestückchen mit Sauerstoff zu versorgen, nach der Formel von Warburg[8].

$$d = \sqrt{8 \cdot C_0 \frac{D}{A}}$$

Wobei: D = Diffusionskonstante. Wir verwendeten hier den einzigen uns bekannten Wert für pflanzliches Material, der von Wanner[9] an Keimlingswurzeln von *Allium cepa* gemessen wurde. A = Sauerstoffverbrauch des Gewebes pro Volumen- und Zeiteinheit. C_0 = Sauerstoffkonzentration an der Schnittgrenze.

Man findet so: Sauerstoffüllung der Gefässe: d = 0,35 mm, Luftfüllung der Gefässe: d = 0,16 mm.

Als kleinste Dimension unserer Gewebestückchen können wir die Blattdicke zu etwa ⅓ mm annehmen.

A. M. Landolt

Zürich, Spyristeig 35, 30. Oktober 1957.

Summary

It is demonstrated that the abnormally high respiratory quotient of the green tobacco leaf as described by various authors is due to an insufficient supply of oxygen to the tissues. In pure oxygen the quotient is 1·0.

[8] O. Warburg, *Über den Stoffwechsel der Tumoren* (Berlin 1926).
[9] H. Wanner, Vjschr. naturf. Ges. Zürich *90*, 98 (1945).

4-Chlorotestosterone Acetate and Gonadotrophins

It has been shown that 4-chlorotestosterone acetate is a new steroid which presents high anabolic and low androgenic activity[1,2]; the new compound is devoid of progestative or estrogenic properties[1].

Owing to the possible relations, both chemical and biological, between 4-chlorotestosterone and sexual hormones, it seemed of interest to ascertain its effect on the gonadotrophic secretion.

For this purpose the parabiotic rat technique described by Hertz and Meyer[3] has been followed. The castration of the male partner causes an increased secretion of gonadotrophins which pass across the 'parabiotic barrier' and stimulate the accessory sexual organs of the female partner.

Male and female rats averaging 100 g of body weight of the Long Evans strain were joined in parabiosis. The male partner was castrated on the day of operation. The steroid was injected into the castrated male subcutaneously in dose of 30 γ daily for 11 days.

At sacrifice, the weight of the prostate and seminal vesicles in the male and the weight of the uterus and ovaries in the female were recorded.

The results, summarized in the Table show: (1) 4-chlorotestosterone acetate does not significantly increase the weight of the prostate and seminal vesicles of the castrated male rat, while testosterone propionate at the same dosage level causes a significant rise of these organs; (2) 4-chlorotestosterone acetate does not inhibit the sharp increase of the weight of the uterus and ovaries of the parabiotic female rat. On the contrary, testosterone propionate completely inhibits the weight increase of the uterus and ovaries of the female rat: these organs in fact present the same weight as the organs of normal female rats of the same weight and age.

In order to reject the possibility of a preferential transfer of one steroid across the 'parabiotic barrier', we joined two male castrated rats together with the same technique and injected one animal subcutaneously with 30 γ daily of testosterone propionate for 11 days. This treatment increases the weight of the prostate and seminal vesicles of the injected rat, while it does not modify the sexual organs of the partner parabiotic animal.

V. Clini, G. Baldratti, and G. Sala

Laboratori Ricerche Farmitalia, Milano, November 18, 1957.

Riassunto

Il 4-clorotestosterone acetato presenta alta azione anabolica e scarsa attività androgena, è privo di proprietà estrogene o progestative.

Ricorrendo al metodo della parabiosi nel ratto si dimostra che il nuovo steroide non inibisce la secrezione ipofisaria di gonadotropine.

[1] G. Sala and G. Baldratti, Proc. Soc. exp. Biol. et Med., N. Y. *95*, 22 (1957).
[2] G. Sala, G. Baldratti, and R. Ronchi, Folia endocrin. (in press).
[3] R. Hertz and R. K. Meyer, Endocrinology *21*, 756 (1937).

Effect of 4-chlorotestosterone and testosterone on gonadotrophin effect of parabiotic rats (mean organ weight ± standard errors referred to 100 g body weight)

Treatment	No. of pairs	Male castrated rat		Female rat	
		Prostate mg	Seminal vesicles mg	Uterus mg	Ovaries mg
Controls	8	7·7 ± 1·06	12·3 ± 1·87	149·7 ± 20	76·6 ± 10·4
4-chlorotestosterone acetate	7	12·2 ± 1·8	16·7 ± 0·76	147·8 ± 7·26	110·3 ± 12·6
Testosterone propionate . .	6	47·6* ± 8·13	70* ± 9·2	69·6* ± 17·1	35·5* ± 4·22

* Difference from controls highly significant ($P < 0{\cdot}01$).

Hematological Effects of Lathyrism[1]

In connection with other experiments on lathyrism we noticed a high hemoglobin level in the blood. Since the synthesis of collagen (especially of hydroxyproline) is decreased and the relative concentrations of serum proteins reversed[2], a hematological study on the cell maturation and hemoglobin synthesis seemed warranted with reference to the unknown way of action of the toxic agent (β-aminopropionitrile). In the literature we found only a passing note on the polycythemia in lathyrism[3].

The rats were kept for 2 months on a diet containing 56·0% of sweet pea[4]. Hemoglobin was determined with the photometer at 5500 Å after dilution 1:200 with 0·1% sodium carbonate. The white and red cells were counted by standard methods in Buerker's chamber. The bone marrow was studied from smears (stained with May-Gruenwald-Giemsa-solution) obtained from the thighbones. Usually one thousand (in few samples 500) cells were counted.

From the marrow following comparison was obtained.

Table II

	Lathyrus-group	Control-group
	%	%
No. of animals	4	7
Proerythroblasts	1·0	–
Erythroblasts	12·5	7·0
Normoblasts	45·0	42·7
Erythropoesis: total	*58·5*	*49·7*
Myeloblasts	–	0·3
Promyelocytes	0·4	1·8
Myelocytes	14·4	15·6
Matured neutrophils	12·6	15·1
Eosinophils	4·1	7·3
Lymphocytes	6·9	7·1

To conclude, β-aminopropionitrile seems not to affect the division or maturation of the cells nor the synthesis of hemoglobin. This is in agreement with the histological findings on the connective tissue. These symptoms seem to be due to a chronic nitrile intoxication and not directly connected with the characteristic symptoms of lathyrism, which depend on an available amino group[5].

[1] This work forms a part of a program which has been aided by Sigrid Juselius' Stiftelse. The help is gratefully acknowledged.

[2] Unpublished experiments.

[3] B. J. Geiger, H. Steenbock, and H. T. Parsan, J. Nutr. *6*, 427 (1933).

[4] The animals and diet are described in L. Kalliomäki, Maija Yli-Pohja, and E. Kulonen, Exper. *13*, 495, footnote 2 (1957).

[5] T. E. Bachhuber, J. J. Lalich, D. M. Angevine, E. D. Schilling, and F. M. Strong, Proc. Soc. exp. Biol. Med. *89*, 294 (1955).

If the β-aminopropionitrile would compete with β-alanine, symptoms of pantothenic acid deficiency, including anemia, were to be expected[6].

Maija Yli-Pohja,
Taina Tuominen, and E. Kulonen

Department of Medical Chemistry, University of Turku (Finland), August 5, 1957.

Zusammenfassung

Bei experimentellem Lathyrismus wurde eine deutliche Vermehrung von Erythrozytenproliferation und Hämoglobinsynthese beobachtet. Hämoleukozyten und Myelopoese im Knochenmark wurden nicht beeinflusst.

[6] Anemia is found in human lathyrism, which seems to be a different condition. C. Gopalan, Trans. R. Soc. trop. Med. Hyg. *44*, 333 (1950).

5-Hydroxytryptamine in Mammalian Thyroid Gland[1]

Thyroid glands of rats, sheep, rabbits, and dogs were examined for 5-hydroxytryptamine (5-HT). Considering the great quantities of 5-HT present in gastrointestinal mucosa[2], it is not surprising that high concentrations of 5-HT were found in mammalian thyroid glands, which phylogenetically are derived from the phyrangeal epithelium. Surprisingly, however, there are great differences between the 5-HT content of the thyroids of different species.

The tissues were extracted with 90% v/v acetone; otherwise the procedure suggested by Amin *et al.*[3] was followed. The glands were taken from rats and rabbits killed by a blow on the head and from dogs bled to death under ether anesthesia. The sheep thyroids were obtained from the slaughterhouse, where they were immediately put on dry ice. In most cases the cut tissues were left in acetone for a few days at −15°C, and the rest of the extraction was done just before the assay. 5-HT was assayed biologically by using the isolated heart of *Venus mercenaria* in natural sea water with 6 mg/l of benzoquinonium chloride (generously supplied by Sterling Winthrop Research Institute).

[1] This work was supported by a grant from the U.S. Public Health Service (H-2205).

[2] V. Erspamer, Pharmacol. Rev. *6*, 425 (1954).

[3] A. H. Amin, T. B. B. Crawford, and J. H. Gaddum, J. Physiol. *126*, 596 (1954).

Table I

	Hemoglobin g%		Erythrocytes mill./mm³		Mean hemoglobin	Leukocytes in mm³
	Mean	Range	Mean	Range	Mean	Mean
Lathyrus-group (8)	14·5	(13·1 – 16·4)	7·44	(6·60 – 8·01)	19·5	8690
Control-group (8)	12·9	(12·4 – 14·1)	6·25	(5·73 – 6·77)	20·7	8860
P of chance occurrence of the difference	< 0·01		< 0·001		> 0·10	–

The differentiation of the leukocytes was similar in both groups.

The test conditions were described previously[4]. 5-HT was used as creatinine sulfate (generously supplied by Abbott Laboratories); the doses refer to the base.

5-Hydroxytryptamine in Mammalian Thyroid Glands

									Mean µg/g*
Rat	2·5	2·6	2·4	3·3	5·0	5·0	3·9	4·6	3·7
Sheep	3·5	5·7	7·5	3·8	2·4				4·6
Dog	0·025	0·018	0·038	0·02					0·025
Rabbit	0·29	0·09							0·19

* Wet weight.

The 5-HT equivalents are presented in the Table. The concentrations found in the rat and sheep thyroids were more than hundred times those found in the dog thyroids. Rabbit thyroids contained more than those of dogs. In order to ascertain the specificity of these findings, the following studies were carried out.

(1) Ascending paper chromatograms were run using the rat and sheep thyroid extracts in the upper organic phase of *n*-butanol/glacial acetic acid/water (4:1:5). When pieces of the paper strips were eluated and tested on *Venus* heart, the active material was found to travel like 5-HT. When paper chromatograms of sheep thyroid extract were treated according to Jepson and Stevens[5], a fluorescing spot was found at the same site as the spot of the reference 5-HT.

(2) Parallel assays were done with rat and sheep thyroid extracts on the atropinised estrus rat uterus[3] and *Venus* heart. Sheep extracts gave about one third lower results on the uterus. The reason for this is supposed to be the presence of catechol amines, which relax the uterus. In one sheep thyroid gland 0·2 µg/g of norepinephrine and 0·03 µg/g of epinephrine were found when analysed according to v. Euler[6]. The rat extracts, on the other hand, were about 30% more active on the uterus than on the heart. LSD treatment did not completely abolish the response of the uterus to rat thyroid extracts, although it blocked the effect of 5-HT. There was little, if any, sign of this LSD-resistant activity in sheep and dog thyroid extracts. The low amount of 5-HT in the dog thyroid gland was also confirmed on the uterus. The amounts of norepinephrine and epinephrine in one dog thyroid were 0·3 and 0·04 µg/g respectively.

(3) That the activity measured on *Venus* heart was due to 5-HT is strengthened by the similar time course of the response. After washing, the effect of bufotenine, and, to a much lesser degree, the effect of N-methyl-5-HT, lasts longer than the effect of 5-HT. The molluscan heart is resistant to most substances likely to have an effect on smooth muscle[7].

(4) The rat thyroid extract did not lose its activity on the *Venus* heart when boiled for 10 min in 1 *N* HCl, while the same treatment in 1 *N* NaOH destroyed about 80%.

(5) Reserpine (5 mg/kg intraperitoneally) was not able, within 4 h, to decrease significantly the *Venus* heart activity of rat thyroid glands; however, neither was their a clear-cut 5-HT depletion in the duodenum of these rats.

For comparison some salivary glands were extracted and their activity tested on the *Venus* heart. In the rat rather high values were found (in µg/g, parotid: 0·68, 0·44; submaxillary: 0·19, 0·31; sublingual: 0·61, 1·0; extraorbital lacrimal: 0·34, 0·26). In the dog, the values were again lower (parotid: 0·03, 0·005; submaxillary: 0·02, 0·004); and in the rabbit, they were between those of the rat and the dog (parotid: 0·16; submaxillary: 0·13).

Conclusions: High 5-HT values were found in rat and sheep thyroid glands and very low values in dog thyroids. In the rat thyroid, there appears to be an unidentified uterus-stimulating substance which is soluble in 90 % acetone. On rat uterus, it is atropine-resistant. It is not histamine, because the tissues used for analysis are resistant to this amine. Garven[8] tested two acetone extracts of rabbit thyroid on the rat uterus and found one of them to produce an LSD-resistant response. The smooth muscle-stimulating substance of Koepf and Mezen[9], which is present in rat salivary glands, also was found to contract the rat uterus. It is not known where 5-HT is localized in the mammalian thyroid gland. The mast cells are a possible site, since they are known to contain this amine[10]; certain cells of the enterochromaffin type are another possible location. It is improbable that 5-HT has anything to do with the specific thyroid function.

It is a pleasure to thank Professor O. Krayer for making my visit to his department possible and for his interest in this work. Helpful advise by Professor V. Erspamer is gratefully acknowledged.

M. K. Paasonen*

Department of Pharmacology, Harvard Medical School, Boston, Mass., September 13, 1957.

Zusammenfassung

Schilddrüsen von Ratten und Schafen enthalten relativ grosse Mengen von 5-Hydroxytryptamin. In der Thyreoidea von Hunden wird dieser Stoff nur in einer hundertmal kleineren Konzentration vorgefunden. Weiter wird gezeigt, dass in der Rattenthyreoidea eine nicht identifizierte, acetonlösliche Substanz vorhanden ist, welche Uteruskontraktionen auszulösen vermag.

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Fat Metabolism in Experimental Obesities VIII. Blood Total Lipids and Ketones in Four Kinds of Obese Mice*

To our knowledge, no systematic study has been conducted of blood lipids and ketone levels in various

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Table I

Blood Glucose of Non-Obese Mice and of Mice with Four Different Obesities (mg%)

	Hereditary Obese-Hyperglycemic and Controls		Goldthioglucose Obese and Controls		Yellow Obese and Controls		Adrenotropic Tumor Obese and Controls	
	Obese	Non-Obese	Obese	Non-Obese	Obese	Non-Obese	Obese	Non-Obese
Fed – No treatment . .	286 ± 93	127 ± 10	157 ± 31	114 ± 21	151 ± 32	130 ± 12·1	108 ± 10	128 ± 3
After 18 h fast	128 ± 36	62 ± 11	97 ± 13	87 ± 18	78 ± 29	85 ± 24	115 ± 13	77 ± 8
After 0·5 units Insulin	215 ± 90	35 ± 14	49 ± 12	52 ± 9				
After 2 × 2 mg growth hormone	382 ± 60	125 ± 18	167 ± 42	134 ± 17				

experimental obesities. Previous studies published in this series (e.g.[1]) and elsewhere (reviewed in [2]) have emphasized the differences between 'regulatory' obesities, characterized by central lesions in the mechanism regulating food intake, and 'metabolic' obesities, characterized by primary disturbances in fat metabolism. For instance, in the first type (exemplified by surgically-induced and by goldthioglucose-induced hypothalamic obesity) increased lipogenesis takes place only in the measure in which an increase in food intake is allowed. In the second type (exemplified by the obese hyperglycemic syndrome and by the obesity due to ACTH-secreting tumors), lipogenesis is increased over the normal even if the obese animals are paired-fed with their non-obese controls or if both groups are fasted[3]. In turn, different metabolic obesities will differ in the nature of the primary metabolic abnormality. For instance, obese-hyperglycemic mice have very different endocrine, enzymatic, metabolic and behavioral characteristics from ACTH-obese mice[2]. It appeared of interest to see whether the blood lipid and ketone picture would show a similar diversity.

Materials and Methods.—The mice used belong to four kinds of obesity available at this time for this species in our laboratory[4]. Mice with the recessive hereditary obese-hyperglycemic syndrome[2], ranged in age from 3 to 7 months, weighed from 40 to 90 g for the obese, 19 to 33 g for their non-obese littermates. No differentiation is made in the tables of results on the basis of sex for these and other types of mice as no difference was detected between males and females as regards total lipids or ketone bodies. Goldthioglucose hypothalamic obese swiss mice[5] had been successfully injected with 1 mg/g of goldthioglucose 3 to 5 months prior to the experiment. Controls were swiss mice unsuccessfully injected or left uninjected, no difference being detected between the two groups. Obese and non-obese mice in the goldthioglucose group were 7 to 9 months of age; the obese animals weighed 50 to 70 g, non obese 30 to 40 g. Adrenotropic-tumor bearing mice[6], were six months old and had received the tumor transplant two months previously. The 'obese' animals weighed on the average 30 g, the non obese 27 g (in this type, there can be considerable increase in proportion of bodyweight represented by fat without any marked increase in weight[7], in other words 'obesity' is present even in the absence of 'overweight'). ACTH animals, being highly susceptible to infections, were given terramycin in their drinking water (concentration 2 g per 1000 cm^3). Mice with the dominant heterozygous yellow obesity[8] were 4–6 months of age and weighed 40 to 60 g; their non-obese littermates weighed 24 to 30 g. All animals were housed in pairs (one obese, one non-obese) in small cages for at least two weeks preceding the period when determinations were made and throughout that period they received food and water *ad libitum* except for 'fasting' experiments when the animals were deprived of food 18 h prior to the determination. In addition, in the obese hyperglycemic mice, as detailed studies had previously been conducted in lipogenesis following the administration of various hormones to these animals[9], the effects of growth hormone and of insulin on total lipids and ketone levels were also determined. The insulin was given intraperitoneally, a dose of 0·5 units being given in 0·25 cm^3 aqueous solution, the samples being taken 30 min after the administration of the hormone. Growth hormone was also given intraperitoneally, each dose of 2 mg being dissolved in 0·25 cm^3 of saline at a pH of 8·5. Each mouse received two doses in two consecutive days, the samples being drawn 15 h after the second injection. Each animal acted as its own control, with no less than 10 days elapsing between experiments. Blood samples were drawn from the tail vein. Glucose levels were determined by the Somogyi-Nelson micromethod[10]. The blood lipid levels were determined by the turbidimetric method of de la Huerga, Yesneck, and Papper[11]. Ketone bodies were determined as acetone and acetoacetic equivalent by the colorimetric method of Greenberg and Lester[12].

Results and Discussion.—Table I gives blood glucose levels of the animals under study and confirms previous findings on blood glucose of these various types of obese

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Table II

Blood Total Lipids, Acetone and Aceto Acetic Acid Equivalent of Non-Obese Mice and of Mice with Four Different Obesities

Type of Animal and Treatment	Total Lipids		Acetone		Aceto Acetic Acid	
	Obese	Non-Obese	Obese	Non-Obese	Obese	Non-Obese
Obese Hyperglycemic Fed — No Treatment	955 ± 239 (12)	632 ± 148 (12)	3·17 ± 1·49 (15)	2·03 ± 0·79 (15)	3·48 ± 1·64 (15)	3·25 ± 0·88 (15)
Obese Hyperglycemic Fasted 18 h	1255 ± 364 (10)	1105 ± 259 (8)	2·30 ± 1·37 (15)	3·59 ± 0·40 (13)	2·53 ± 1·03 (13)	3·75 ± 0·47 (13)
Obese Hyperglycemic ½ I. U. Insulin per Mouse	1221 ± 114 (10)	1246 ± 236 (8)	2·57 ± 1·38 (10)	2·99 ± 2·11 (9)	2·82 ± 1·50 (10)	3·29 ± 2·29 (9)
Obese Hyperglycemic 2 mg Growth Hormone per Mouse for 2 Days	1598 ± 199 (9)	1631 ± 82 (6)	3·28 ± 1·99 (11)	2·85 ± 1·59 (10)	3·60 ± 2·31 (11)	3·13 ± 1·74 (10)
G. T. G. Fed	1214 ± 55 (6)	872 ± 71 (6)	1·70 ± 0·38 (16)	1·50 ± 0·25 (13)	1·87 ± 0·31 (16)	1·64 ± 0·28 (13)
G. T. G. Fasted 18 h	1756 ± 32 (6)	1313 ± 47 (6)	1·94 ± 0·61 (15)	2·18 ± 1·01 (10)	2·13 ± 0·67 (15)	2·32 ± 1·10 (10)
Yellow Fed	1517 ± 76 (8)	1174 ± 57 (6)	3·68 ± 1·54 (16)	1·39 ± 0·39 (11)	4·05 ± 1·68 (16)	1·53 ± 0·44 (11)
Yellow Fasted 18 h	1763 ± 92 (8)	1597 ± 64 (6)	2·04 ± 0·66 (11)	1·97 ± 1·15 (10)	2·28 ± 0·79 (11)	2·14 ± 1·16 (10)
A. T. T. Fed	1806 ± 50 (5)	1397 ± 134 (5)	1·24 ± 0·13 (6)	1·49 ± 0·40 (4)	1·36 ± 0·18 (6)	1·64 ± 0·53 (4)

animals (reviewed in [2]). The mice with the hereditary obese hyperglycemic syndrome show a very much elevated blood glucose—in this series over twice the level of the non-obese. This high level is relatively little affected by insulin, further elevated by growth hormone. By contrast, the blood glucose level of goldthioglucose obese mice, like that of normal mice, is almost unaffected by growth hormone under the conditions of the experiment, and decreased to such levels by insulin that convulsions appear in some of the animals. The non-fasted glucose level of yellow mice is elevated in some of the animals. Unlike that of normal mice the blood glucose level of mice bearing adrenotropic tumors is relatively unaffected by fasting.

Table II gives the blood lipids of the various groups of animals. It is of interest that the four strains of non-obese mice have markedly different levels of blood total lipids. On the other hand, all these animals react similarly to fasting: as could be predicted, fasting elevates lipid levels; in conformity with classical results, insulin and growth hormone cause an increased lipemia in the thin (normal) littermates of the hereditarily obese-hyperglycemic mice. In each of the four types of mice studied, blood total lipid levels are higher in the obese than in the non-obese mice. In the three types of obese mice in which the effect of fasting was determined, it led to a significant increase in total blood lipids. In the obese hyperglycemic mice, the various treatments tend to make levels similar to these of the control animals.

Of particular interest are the results concerning blood acetone and aceto-acetic acid levels. In the non-obese animals, in accordance with classic results, fasting causes a highly significant increase in ketone levels. The goldthioglucose obese mice (exemplifying 'regulatory obesity') behave as do normal animals and exhibit increased ketone levels in fasting. By contrast, in the hereditarily obese-hyperglycemic mice (exemplifying 'metabolic obesity') fasting decreases ketone levels. It thus appears that reaction of ketone levels to fasting may provide an additional method of distinguishing between 'regulatory' and 'metabolic' obesities. Yellow obese animals follow a pattern similar to that exhibited by the obese hyperglycemic mice, blood ketone levels being cut in half by fasting as compared to the expected rise in the non-obese littermates.

In connections with the last finding it may be relevant to recall that while the blood cholesterol level of goldthioglucose obese mice is normal[2], that of mice with the obese-hyperglycemic syndrome[13], of mice made obese by ACTH secreting tumors[14] and of yellow obese mice[15] is elevated by amounts varying from 30% in the case of the yellow mice to 100% in the case of the obese hyperglycemic mice and the ACTH mice. The results of this study again point to the fact that, while there are certain non-specific results of hyperphagia and obesity (e.g. elevated serum lipids) there are also profound differences between regulatory obesity, with its near normal metabolic pattern, and metabolic obesities with their galaxies of abnormalities primary to the various syndromes (e.g. blood glucose picture, enzymatic activities, hormonal patterns, cholesterol metabolism and, in this study, the effect of fasting on blood ketones).

J. Mayer and D. J. Silides

Harvard University, School of Public Health, Boston (Massachusetts), October 15, 1957.

Résumé

Chez la Souris, l'obésité «de régulation» (ici: l'obésité hypothalamique due à l'aurothioglucose) comme les obésités «de métabolisme» (exemples: syndrome récessif d'obésité-hyperglycémie, syndrome dominant d'obésité «jaune», obésité due aux tumeurs secrétant l'ACTH) sont accompagnées d'hyperlipémie, accentuée par le jeûne.

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Par contre si, comme les souris normales, les souris obèses hypothalamiques réagissent au jeûne par une augmentation de la cétonémie, les souris héréditairement obèses réagissent au jeûne par une diminution de la cétonémie, une réaction qui peut s'ajouter à celles qui différencient les obésités «de métabolisme» des obésités «de régulation».

Nuclear Chimaeras in the Newt

Direct tests as to whether the cell nuclei of various tissues differ in their developmental capacities have been made recently by Briggs and King. These authors developed a technique for the transplantation into frog oocytes of somatic nuclei from embryos of various stages. The recipient oocytes are first enucleated and artificially activated, and the degree of development they reach after nuclear transfer shows the capacity of implanted nuclei to carry the oocyte cytoplasm towards normal differentiation. The above authors have reviewed their investigations recently[1]; whilst blastula nuclei, including nuclei from the dorsal lip of the blastopore, are capable of bringing about the normal development of enucleated oocytes, endoderm nuclei from late gastrula show definite signs of differentiation in that a high percentage of them are incapable of effecting development beyond the gastrula stage.

It has been found difficult to carry out parallel investigations with the oocytes of newts, as these cannot become artificially activated and are normally polyspermic. Attempts made so far[2] involved substantial modifications of the Briggs and King procedure, and the results have not been entirely satisfactory.

It might be of interest to record here a further experiment carried out on *Triturus alpestris* material.

A group of 2 to 4 somatic nuclei were transplanted into a normally fertilized and non-enucleated egg. As a result, this egg carried its normal zygotic nucleus as well as the donor nuclei. It was hoped that the hosts would develop to a more or less advanced stage, at which it would be possible to observe whether the donor nuclei are able to participate in tissues derived from all three cell layers or only in tissues derived from the cell layer of their origin.

To make identification of implanted nuclei possible, haploid embryos were used as donors. These were produced by Dr. G. G. Selman of this Institute, who treated *in vitro* newly collected sperm with ultraviolet light of 2100–3200 Å for 1·5 to 3 min. The sperm was then smeared onto oocytes collected from the oviduct. The donors were checked for haploidy by chromosome counts on squashes from a piece of tissue.

The host eggs were left to develop after the nuclear transfer. Sixteen were fixed at the early neural plate stage, sectioned and stained with Feulgen for chromosome counts. The remaining failed to develop normally beyond the early neural plate stage, became abnormal and finally cytolysed.

Examination of the 16 specimens fixed showed that no haploid nuclei could be seen in 5; but several haploid (donor) as well as diploid (host) nuclei were found in each of the remaining 11. Of these 11, 7 had received haploid ectodermal nuclei from the neural plate itself of donor embryos whilst 4 had received nuclei from the chorda mesoderm of the same donors.

It is interesting that, in almost all of the eleven cases, nuclei of donor origin were found in all three cell layers. Thus, not only the embryos as a whole but also each cell layer were nuclear chimaeras, containing nuclei of two distinct origins and chromosome complements, within cytoplasm of one origin.

Sizes of adjacent haploid and diploid cells were markedly different. Incidentally, several tripolar spindles and aneuploid nuclei were also observed.

It follows that at the definite neural plate stage, the nuclei of the mesoderm and the neural plate itself are all capable of organizing cells of any cell layer and are not differentiated at least in this respect. As all hosts failed to complete development, it cannot be decided whether these nuclei differ in their capacity for further tissue differentiation.

In another series, ectodermal nuclei from the neural plate of diploid hosts were transplanted into haploid donors. This series is of less interest than the first one because the nuclei of haploid hosts might be expected in some cases to become diploid and thus be confused with donor nuclei; furthermore, haploid embryos are expected to be more prone to abnormalities. Nine hosts were fixed in this series, 7 as gastrulae and 2 as blastulae. In 3 no reliable chromosome counts could be made. Again, haploid and diploid cells were found in all cell layers of the other four.

As already indicated, the recipients that were not fixed at the early neural plate stage developed abnormalities and cytolysed soon afterwards. It is hoped however that these abnormalities might be avoided or delayed in further tests by: (a) implanting one donor nucleus only instead of a cluster of 2 to 4, and (b) grafting tissues from the chimaeras onto healthy normal embryos, where these tissues might differentiate further.

We wish to thank Professor C. H. Waddington for his interest in this work and Dr. G. G. Selman for producing the haploid embryos used. We are thankful to the Melville Trust (E.M.P.) and the Royal Commission for the 1851 Exhibition (J.J.) for Research Fellowships.

E. M. Pantelouris and J. Jacob

Institute of Animal Genetics, Edinburgh, October 28, 1957.

Zusammenfassung

Gruppen von 2–4 somatischen Kernen haploider *Triturus*-Embryonen wurden in diploide, ungefurchte Eier injiziert. 11 haplo-diploide Mosaik-Larven entwickelten sich bis zum frühen Neurula-Stadium. Nachkommen von Kernen aus dem neuralen Ektoderm oder Chorda-Mesoderm von Spendern im Neurula-Stadium fanden sich in allen drei Keimblättern der Wirtslarve.

The Temporary Inactivation of Newt Larvae by Benzimidazole and its Alkyl Derivatives

Benzimidazole and some of its derivatives, produce muscle relaxation in mammals, apparently by their action on the central nervous system[1]. The experiments

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reported here show that similar effects are produced in newt larvae by benzimidazole itself and, more effectively, by certain of its alkyl derivatives.

Experimental.—A commercial preparation of benzimidazole was used. The alkyl derivatives were prepared in the laboratory[2].

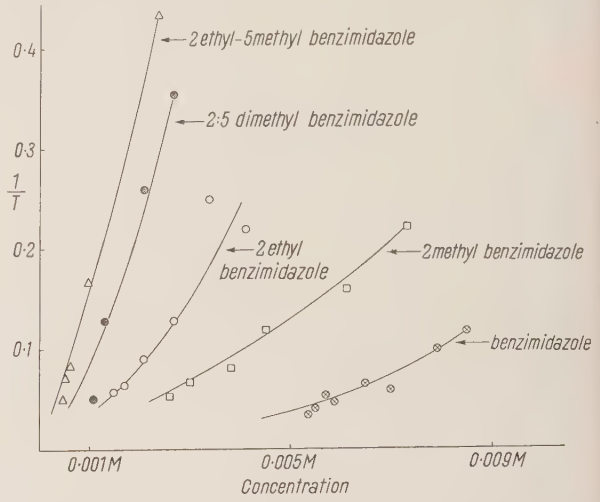
These experiments were performed on larvae of *Triturus alpestris* which had reached a stage of development characterized by the appearance of a third digit on the fore limb. (Comparable with Glaesner's stage 41–42 for *T. vulgaris*[3].) Single larvae were placed in about 10 ml of dilute solutions of benzimidazole and of its 2-methyl, 2-ethyl, 2:5 dimethyl and 2 ethyl-5 methyl derivatives. The solutions were prepared in 1/10th Holtfreter solution and the pH was adjusted, if necessary, to 7·0–7·2 with dilute hydrochloric acid. The concentrations of the benzimidazole used ranged from 70 to 1000 μg/ml.

Results and Discussion.—On placing in the benzimidazole solutions the larvae at first showed increased activity. After a time swimming movements became less frequent, the animals occasionally swimming upside down. Finally the animals became quiescent and no longer responded to contact stimuli. The animals were considered to be completely inactivated when they remained on their backs for at least 2 min after being placed in this position. When returned to 1/10th Holtfreter solution the animals usually responded to contact stimuli within 5 to 10 min, and thereafter behaved normally. The time taken to reach the arbitrary state of inactivation depended on the concentration and nature of the benzimidazole used. After treatment the animals appeared to suffer no ill effects of any kind. Larval development was normal. Most of the animals were alive at least two months after the completion of the experiments.

The results of the experiments are summarized in the Figure. In this graph the reciprocal of the time taken to reach the given inactive state is plotted against the concentration of the benzimidazole employed. In most cases each point represents the mean of four observations. The experiments were performed at a temperature of 20–23°C.

The behaviour of the larvae when placed in the benzimidazole solutions, and their rapid and apparently complete recovery when removed from these solutions, suggests that the compounds exert a depressant action on the nervous system. It is not possible to decide whether the compounds act centrally and produce a muscle relaxation analogous to that seen in mammals, or whether a general narcosis, such as that caused by urethane, is produced.

The results show that newt larvae are more readily inactivated by certain alkyl derivatives of benzimidazole than by benzimidazole itself. The compounds become more effective in order of increasing molecular weight. The alkyl derivatives of benzimidazole are also more effective than the parent compound in producing abnormalities in chick and amphibian embryos[4] and in inhibiting the growth of influenza virus on chick allantois[5]. In particular, in these systems, the 2 ethyl-5 methyl derivative is some 10 to 20 times more potent than the unsubstituted benzimidazole. From the graph, shown in the Figure, it may be calculated that 2 ethyl-5 methyl benzimidazole is about 12 times more active than benzimidazole in depressing the activity of newt larvae.



Alkyl substitution of benzimidazole will give an increased molecular volume, increased basic strength[6], and almost certainly an increased solubility in lipid relative to water. Any of these changes could give rise to the increased potency of the alkyl derivatives. In the case of the 2 ethyl-5 methyl derivative, however, there is a striking coincidence between the increased ionization brought about by alkyl substitution and in the effectiveness of this compound in halting temporarily, or permanently, certain biological processes. Using the method described by Albert[7] it may be calculated that at pH 7·0 the 2 ethyl-5 methyl derivative is about 17 times more ionized than is the parent compound. This corresponds, fairly well, to the magnitude of its increased activity, compared with benzimidazole, against diverse biological systems. The evidence suggests that the cationic form of the benzimidazole is essential for these compounds to exert a toxic or narcotic action. This view, however, cannot be completely reconciled with the facts presented by Slonimski[8] which suggest that only the unionized form of benzimidazole is effective in preventing the growth of yeast cells.

Acknowledgements.—I wish to thank Professor C. H. Waddington for the interest he has shown in this work. I am also indebted to Dr. Louis S. Goodman (University of Utah) for providing some key references. The work was assisted by a grant from the Advisory Committee for Medical Research for Scotland.

F. Billett

Institute of Animal Genetics, University of Edinburgh, November 12, 1957.

Zusammenfassung

Benzimidazol und einige von seinen Alkylderivaten inaktivieren Molch-Larven schon in kleinen Konzentrationen. In verdünnte Holtfreter-Lösung zurückgebrachte Tiere gewinnen ihre normale Aktivität schnell wieder. Der zeitweilige Beweglichkeitsverlust scheint analog dem bei Säugetieren durch Benzimidazol erzeugten.

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Etude sur la pathogenèse de l'œdème au dextrane chez le rat[1]

L'observation de MOELLER[2] selon laquelle la néphrectomie bilatérale protège le rat contre les effets toxiques du dextrane a fait le sujet d'une étude récente publiée par SELYE[3]. Celui-ci a démontré que l'extirpation des reins empêchait la réaction œdémateuse d'apparaître seulement en presence des surrénales. En effet, l'animal surrénalectomisé et néphrectomisé réagit au dextrane tout comme l'animal intact. Cette étude, toutefois, n'amène pas d'éclaircissement nouveau sur la pathogénèse de l'œdème au dextrane; la surrénale peut changer le seuil de réactivité de l'animal sans nécessairement être concernée dans le développement de l'œdème. Au reste, la susceptibilité du rat surrénalectomisé pour le dextrane peut être corrigée par des agents autres que les hormones cortico-surrénaliennes[4].

Une méthode qui se prête bien à l'étude de ce problème est la désensibilisation progressive de l'animal par l'administration d'histamino-libérateurs[5]. Il suffit en effet de quelques jours de traitement avec du 48/80 ou encore avec de la polymixine pour rendre le rat résistant à l'action du dextrane[6]. Ceci proviendrait d'une baisse importante dans la teneur des tissus en histamine et en sérotonine[7].

Il nous a paru intéressant d'utiliser cette méthode afin de vérifier jusqu'à quel point la surrénale est indispensable pour prévenir la réaction au dextrane après néphrectomie bilatérale. Nos résultats démontrent qu'un traitement préalable avec un histamino-libérateur peut empêcher l'apparition de l'œdème, même en absence du rein et de la surrénale.

Notre arrangement expérimental ainsi que les résultats obtenus figurent au Tableau. Quarante rats femelles Sprague Dawley, d'un poids moyen de 135 g (variation extrême ± 5 g), furent partagés en quatre groupes égaux. Ils reçurent la diète ordinaire de Purina Fox Chow et de l'eau comme boisson. Les animaux du Groupe IV furent traités au 48/80 pendant sept jours. Ce libérateur d'histamine était administré par voie souscutanée, en solution physiologique; la dose quotidienne était donnée en deux injections. Une dose initiale de 200 mcg a été accrue de 200 mcg par jour jusqu'à un total de 1 mg, maintenu pendant les trois derniers jours. On pratiqua ensuite les néphrectomies et les surrénalectomies. Les animaux des Groupes III et IV, surrénalectomisés et néphrectomisés, reçurent une injection souscutanée d'une suspension microcristalline de cortisol et de DOCA à raison de 400 mcg chacun. Cette thérapeutique de substitution s'est avérée suffisante pour maintenir les animaux en bon état général durant l'expérience sans modifier par ailleurs la réponse au dextrane.

24 h après les interventions, on injecta, par voie intrapéritonéale, 0,3 ml d'une solution 3% de dextrane (Abbott) à tous les animaux. L'œdème qui apparut au niveau des pattes et du museau fut apprécié d'heure en heure selon une échelle arbitraire de 0 à +++. La moyenne des lectures de la deuxième heure est consignée au tableau des résultats.

Réaction au dextrane chez le rat surrénalectomisé et néphrectomisé après un traitement au 48/80

Groupe	Traitement	Moyenne des lectures de l'œdème*	
		Incidence (%)	Intensité
I	—	100	+++
II	Néph-X.	40	+
III	Néph-X + Surr-X . . (DOCA + Cortisol) .	70	+++
IV	Néph-X + Surr-X + 48/80 (DOCA + Cortisol)	0	0

* 2 h après l'injection au dextrane.

Nos résultats confirment à la fois les observations de MOELLER et de SELYE (Groupes II et III). Toutefois, les animaux du Groupe IV, prétraités au 48/80 et également néphrectomisés et surrénalectomisés sont demeurés insensibles au dextrane. Nous pouvons donc conclure que ni la surrénale, ni le rein ne sont directement concernés dans cette réaction.

Bien que le mode d'action du 48/80 ne soit pas encore entièrement élucidé, il a été démontré que l'injection répétée d'un histamino-libérateur abaisse considérablement le taux d'histamine normalement très élevé au niveau de certains organes comme les pattes et le museau. La réaction d'hypersensibilité au dextrane chez le rat serait donc un phénomène essentiellement lié aux constituants mêmes des tissus réacteurs. Au demeurant, nous avons déjà pu démontrer que la réactivité de ces tissus demeure inchangée même après transplantation[7]. D'autre part, des agents comme la chlorpromazine et la dibénamine peuvent aussi prévenir l'œdème au dextrane chez le rat surrénalectomisé et nous avons observé récemment qu'une modification de la glycémie, à elle seule, change cette réaction[8].

En résumé, l'ablation des reins ou des surrénales n'exerce plus aucune influence sur la réaction au dextrane chez le rat prétraité au 48/80. Il semble donc que les facteurs en cause dans ce phénomène d'hypersensibilité soient inhérents aux tissus récepteurs.

G. JASMIN et P. BOIS

Laboratoire de Recherches, Département d'anatomie pathologique, Université de Montréal (Canada), le 26 novembre 1957.

Summary

Rats bilaterally nephrectomized and adrenalectomized were injected with dextran. It was found that the edematous hypersensitivity reaction which developed in these animals can be prevented by a pretreatment with compound 48/80.

[1] Ces recherches furent exécutées grâce à un octroi de la Fondation Rhéaume de Montréal. Nous remercions les compagnies pharmaceutiques Burroughs et Wellcome Ltd., New York, E.-U., CIBA Ltd., Bâle, Suisse et Pfizer Canada, Montréal, Canada, qui nous ont fourni respectivement le 48/80, DOCA et cortisol.

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The Axon Reflex Sweating Produced by Potassium and Sodium Cyanides

In human skin, sweating is produced through axon reflex mechanism by intradermal injection of nicotine and other agents with nicotinic action[1], and also by sodium chloride and some other sodium salts[2]. The axon reflex sweating can be elicited also in the toe-pads of the cat by nicotine and sodium chloride: this response is abolished by degeneration of the postganglionic sympathetic nerves, but not influenced by degeneration of the sensory nerves supplying the toe-pads[3]. The receptors responsible for this sweating are presumed, for the present, to be specialized parts of the sympathetic sweat nerve endings.

In the present experiments, we investigated the effect of potassium and sodium cyanides on the receptors for the axon reflex sweating and found that the cyanides themselves have a property of provoking the axon reflex response by acting on the receptors.

Observations were made on the toe-pads of nine cats of both sexes, weighing 2·2 to 3·6 kg. The techniques used were the same as those described in our previous paper[3]. In order to exclude sweating of central origin in the toe-pads of the hind feet, the sciatic nerves were sectioned about 4 to 5 h previously under ether anaesthesia. To visualize sweating, the iodine-starch method of Wada and Takagaki[4] was used. For identification of the axon reflex sweating, the band method[2] was applied. The test agents used were dissolved in 0·9% NaCl solution and injected subcutaneously to the toe-pads on the distal side of the band under non-anaesthesia, as illustrated in the Figure.

The effect of KCN on the axon reflex sweat response to nicotine was studied in 4 cats. Injection of 0·05 ml of nicotine in 10^{-4} or 10^{-3} on the first day after section of the sciatic nerves showed an evident axon reflex sweating in all of toe-pads tested. As described in a previous paper[3], the responsiveness of each toe-pad to the axon reflex provoking action of nicotine diminished more or less rapidly, as the days elapsed. On the 5th to 7th day after the sciatic nerve section, the response became doubtful or no longer detectable even with 10^{-2} nicotine.

In most experiments, KCN in 10^{-3} was applied in mixture with nicotine in 10^{-4} to 10^{-2}. No evidence was obtained to indicate that KCN inhibits the axon reflex response to nicotine. Injection of KCN in 10^{-3} alone elicited occasionally a slight sweating around the site of injection. KCN in 10^{-2} alone was tested in the fourth cat on the second day after the nerve operation. It was an unexpected finding that this concentration of KCN produced a vigorous axon reflex sweating.

In 18 out of 23 tests in 6 cats, performed on the first to third day after the sciatic nerve section, the axon reflex sweat response was observed with KCN in 10^{-2}. The failure of the response in 5 tests was proved to be due to the decreased sensitivity of the receptors for the axon reflex. The response commenced usually within 20 to 50 s after the start of injection. Not rarely, over 100 sweat spots were counted on either side of the band. The pattern of the response was quite analogous to that of the axon reflex sweating produced by nicotine. Then, attempts were made to determine the effects on the sweat response to KCN of procaine hydrochloride, atropine sulphate and hexamethonium bromide.

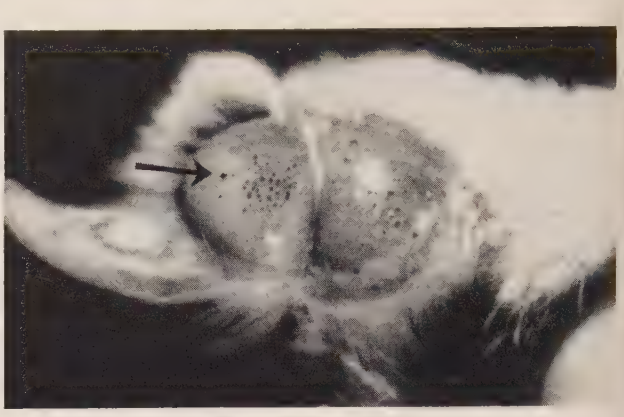

Fig. 1. — The axon reflex sweating on the cat's toe-pad. A band was applied across the middle part of the toe-pad on the second day after section of the sciatic nerve. Subcutaneous injection of 0·05 ml of 10^{-2} KCN (in mixture with hexamethonium) was made to the distal side of the band. Sweating was visualized as black spots. The site of injection is indicated by arrow. White spots on the toe-pad are caused by reflection of light. Magnification, 3·5 ×.

The effect of procaine in 10^{-3} was examined in 13 tests in 5 cats: in all tests the sweat response to KCN in 10^{-2} was completely inhibited. Likewise, the effect of KCN was abolished by atropine in 10^{-3} in all of 12 tests in 4 cats. On the contrary, hexamethonium in 10^{-3} was without any inhibitory effect in all of 22 tests in 5 cats (Figure). These results are very similar to those obtained from the experiments on the axon reflex sweating produced by high concentration of NaCl[5]. This fact supports the view that the axon reflex sweating occurs without synaptic transmission by acetylcholine as in the sympathetic ganglion. The failure to see the axon reflex response after injection of KCN in mixture with procaine or with atropine could not be attributed to the decreased sensitivity of the receptors, since positive results were obtained in most of the tests in which the same concentration of KCN was applied alone or in mixture with hexamethonium to the same toe-pads the following day.

In addition, the effect of NaCN was studied in two cats: injection of NaCN in 10^{-2} also provoked a definite axon reflex sweating. Injection of NaCN in 10^{-3} produced a doubtful response, but a subsequent injection of NaCN in 10^{-2} was fully effective.

Further, the axon reflex nature of the sweating produced by the cyanides was confirmed by observations on human skin, using the band method[2]. Intradermal injections of 0·1 ml of NaCN in 10^{-3} and 10^{-2} were made to the dorsal surface of the forearms of one of us (K.). With NaCN in 10^{-2}, the sweat spots began to appear around the wheal of injection within 40 s after the start of injection and spread very rapidly and widely to the uninjected side beyond the band. The size of the sweating areas reached its maximum, e.g. 37 cm², within 6 min after injection. NaCN in 10^{-3} was also effective, but the extent of the response produced was much smaller.

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[5] M. Wada, J. Invest. Dermat. *23*, 63 (1954). – M. Wada, T. Nakagawa, N. Hanawoka, K. Hatanaka, H. Funato, S. Kanazawa, and N. Morikawa, Arch. int. Physiol. Bioch. *65*, 1 (1957).

The receptors for the axon reflex sweating have a property of being stimulated by nicotine and agents with nicotinic action, in common with the sympathetic ganglion cells and the carotid body chemoreceptors. The similarities and differences between the properties of the latter two structures were comprehensively reviewed by Konzett and Rothlin[6]. The sympathetic ganglion cells have been shown to be selectively paralyzed by the cyanides[7]. Since the work of Heymans *et al.*[8], the cyanides, on the other hand, have been known to stimulate the carotid body chemoreceptors. In this respect, the receptors responsible for the axon reflex sweating resemble the carotid body chemoreceptors.

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Physiological Laboratory, Tohoku University School of Medicine, Sendai (Japan), July 12, 1957.

Zusammenfassung

Subkutane Einspritzung von Kalium- oder Natriumzyanid in die Zehenballen der Katze führt über einen Axonreflex zu merklichem Schwitzen. Dieser Effekt von Kaliumzyanid wird durch Procain und Atropin gehemmt, nicht aber durch Hexamethonium. Auch beim Menschen ruft intrakutan eingeführtes Natriumzyanid eine entsprechende Schweissabsonderung hervor.

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The Influence of Frequency of Stimulation on Synaptic Transmission at Different Temperatures

Cooling of the ganglion causes failure of the nictitating membrane contractions on preganglionic nerve stimulation[1]. In some of our preliminary experiments[2], we noticed that at higher frequencies of stimulation nictitating membrane contractions failed earlier on cooling the ganglion. In this communication we report some further experimental results concerning this effect of different frequencies. In some experiments nictitating membrane contractions were recorded, in others the acetylcholine output was determined.

The superior cervical ganglion of chloralosed cats was perfused in the usual way. The cervical sympathetic trunk was stimulated with square voltage pulses of 1 ms duration at frequencies of 2, 10 and 15 shocks per s. The ganglion was heated or cooled in the way described in one of our earlier papers[3]. The temperature was measured by means of a thermocouple connected to a galvanometer. Nictitating membrane contractions were recorded with an isotonic lever. In experiments in which the acetylcholine output was determined, the post-ganglionic trunk was tied and eserine sulphate (1 : 100 000) added to Locke's solution. Acetylcholine was assayed on blood pressure of eviscerated chloralosed cats.

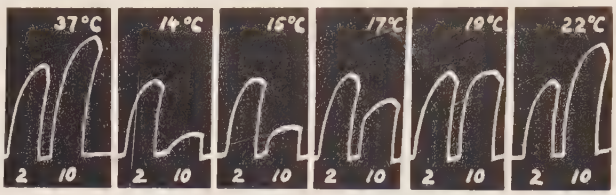

Fig. 1. – Cat, chloralose. Nictitating membrane contractions at different temperatures in the range from 14°C to 37°C. Preganglionic nerve stimulation at 2 and 10 shocks per s.

Figure 1 shows the response of the nictitating membrane to preganglionic stimulation at 2 and 10 shocks per s at 37°C. At 14°C the response of the membrane was considerably more affected for higher rates of stimulation. By increasing the temperature the response to high frequencies gradually returned to its normal value. At temperatures recorded in this experiment (14°, 15°, 17°, 19° and 22°C), the response of the membrane to low frequency stimulation was practically unaffected by temperature changes. These results are in good agreement with those reported by Douglas and Malcolm[4], who found that an inhibition to high frequency of stimulation preceeded the cold block in cat nerves.

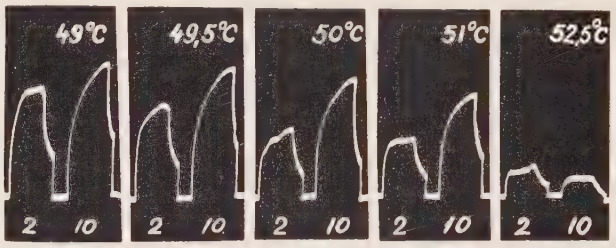

Fig. 2. – Cat, chloralose. Nictitating membrane contractions at different temperatures in the range from 49°C to 52,5°C. Preganglionic nerve stimulation at 2 and 10 shocks per s.

At high temperatures opposite effects were observed. High frequencies of stimulation were more effective. Gradual failure of the membrane contractions to low frequency stimulation started at 49·5°C (Fig. 2). The response to high frequency stimulation was almost unimpaired up to 50·8°C when a steep fall began. At 52·5° C the response to both frequencies was much reduced.

Figure 3 summarises our results. The response of the membrane (on logarithmic scale) has been plotted against the temperature. Two different curves were thus obtained, one for the low frequency (2/s, black dots), the other (circles) for the high frequency of stimulation (10/s). The low frequency curve is shifted to the left in the whole range of temperatures recorded in these experiments.

The influence of frequency of stimulation was also noticable in experiments in which the acetylcholine output was determined at different frequencies. Figure 4 shows the result of four experiments performed at 37°C and at 20°C, respectively. At 37°C the output of acetyl-

[1] K. Kostial, Rad Jug. Akad. Zagreb (in press).

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[3] K. Kostial and V. B. Vouk, J. Physiol. *132*, 239 (1956).

[4] W. W. Douglas and J. L. Malcolm, J. Physiol. *130*, 53 (1955).

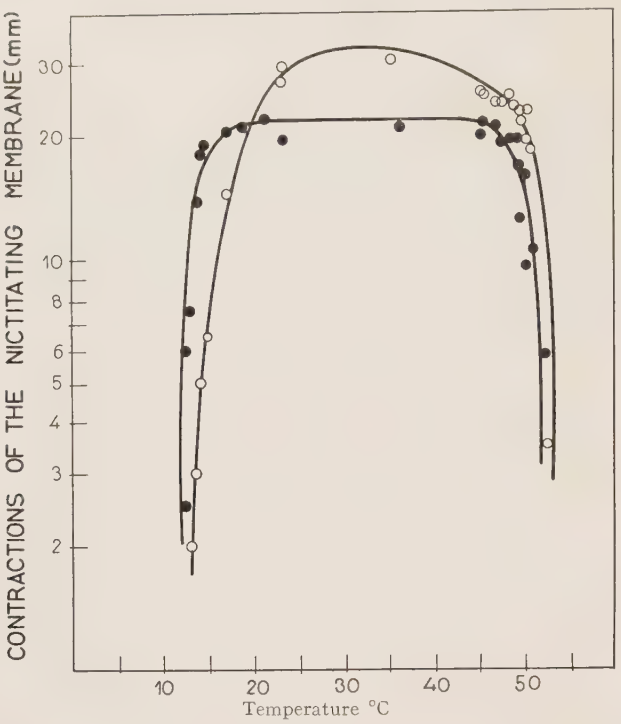

Fig. 3. — Contractions of the nictitating membrane as function of the temperature for two different frequencies of stimulation: 2/s (black dots) and 10/s (white circles).

choline was greatly increased at higher rates of stimulation. When the same experiment was repeated at 20° C an increased number of preganglionic shocks did not increase the total output of acetylcholine.

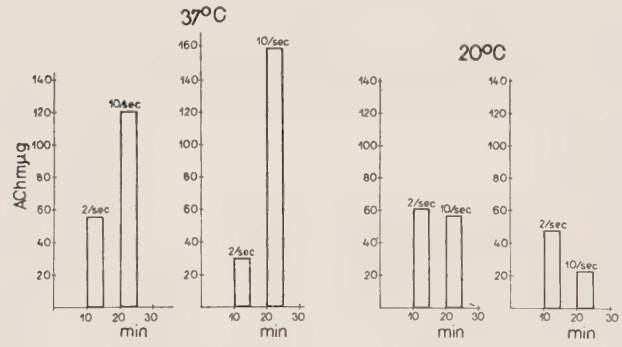

Fig. 4. — Acetylcholine output at 37° C and 20° C for different frequencies of stimulation (2 and 10/s). Each block represents the amount of acetylcholine released during 5 min stimulation of the cervical sympathetic.

These results seem to support our assumption expressed in a previous paper[3] that the disagreement between Browns results[5] and ours was due to the difference in the frequencies of stimulation used in the experiment.

This work has been supported by the Commission for Medical Research (KOMNIS), Beograd.

Krista Kostial and V. B. Vouk

Institute for Medical Research, Yugoslav Academy of Sciences and Arts, Zagreb (Yugoslavia), October 22, 1957.

[5] G. L. Brown, J. Physiol. *124*, 26 P (1954).

Résumé

Durant le refroidissement du ganglion cervical supérieur perfusé du chat, la réaction de la membrane nictitante est notablement plus sensible aux stimulations de haute que de basse fréquence. Aux températures élevées, c'est l'inverse.

L'influence de la fréquence des stimulations a aussi été perceptible lors des expériences, au cours desquelles le rendement de l'acétylcholine a été déterminé. A 37°C, le rendement s'accroît sensiblement lorsqu'on accélère le rythme de stimulation. A 20° C, cependant un nombre croissant de chocs préganglionnaires n'augmente pas le rendement total de l'acétylcholine.

Effects of Intense Noise on Adrenal and Plasma Cholesterol of Mice

The problem of intense noise and its relation to hearing impairment or other systemic injury in animals and man has recently received renewed emphasis. This is partly due to certain legislative measures adopted in some states where hearing loss is now listed as an occupational disease. In part it is also due to the belief that noise is a stressor agent capable of exceeding the limits of physiological hyperactivity of the pituitary-adrenocortical system.

In earlier studies[1] we found that exposure of mice to moderately intense noise (110 db, 10–20 kc) did not result in excessive adrenocortical activation and consequently noise did not appear to constitute a 'harmful' nonspecific stressor stimulus. Criteria used to measure adrenal activation were: adrenal hypertrophy, eosinopenia, thymicolymphatic involution and the appearance of gastric ulcers. Since several workers have reported that noise acts in a fashion similar to other stress agents[2] we decided to extend our studies to include much higher noise levels in an effort to elicit physiological changes characteristic of a stress response.

The sound apparatus used in the present study[3] consisted of a noise generator, a 700 watt University B-24 loudspeaker (having 24 separate 30 watt speakers), a Bogen amplifier and a Navy Beach amplifier. Sound levels attained were about 140 db with most of the energy concentrated in the 150–4800 cy/s frequency range. Such levels approximate those put out by turbo-jet engines and are near the maximal limit one can obtain with conventional diaphragm type loudspeaker systems. Individually isolated mice were exposed to noise for 5 min and autopsied immediately after exposure and at 3 and 6 h intervals for analysis of changes in adrenal weight and in adrenal content of cholesterol and serum Na, K, and Ca levels following noise exposure. Preliminary analyses for total cholesterol were made using the Sperry-Webb technique[4]. This was later abandoned in

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Table I

Cholesterol levels in adrenals and plasma of mice exposed to intense noise

Group	Sex (*n*)	Treatment	0 h		3 h		6 h	
			Adrenal *	Plasma **	Adrenal	Plasma	Adrenal	Plasma
I	M (9)	controls	3·0 ± 0·8	149 ± 9	4·7 ± 0·4	148 ± 4	3·0 ± 0·9	161 ± 10
II	M (15)	sound (114 db, 10–20 kc)	3·5 ± 0·6	146 ± 27	3·7 ± 0·5	146 ± 9	4·5 ± 0·7	150 ± 17
III	M (12)	controls	4·6 ± 1·6	63 ± 7	5·1 ± 0·7	62 ± 13	4·5 ± 0·9	50 ± 16
IV	M (12)	sound (149 db, 150–4800 cy/s)	5·1 ± 1·2	69 ± 2	4·6 ± 1·4	59 ± 8	5·0 ± 1·8	45 ± 11
V	F (12)	controls	7·2 ± 1·9	49 ± 6	6·8 ± 0·4	49 ± 6	8·3 ± 1·8	54 ± 18
VI	F (12)	sound (149 db, 150–4800 cy/s)	7·4 ± 0·7	66 ± 12	6·9 ± 1·0	72 ± 15	8·2 ± 1·0	49 ± 8

* mg cholesterol/100 mg adrenal ** mg cholesterol/100 ml plasma

favor of a simpler method of analysis recommended by Zlatkis, Zak, and Boyle[5].

Cholesterol levels in adrenals and plasma of control mice and mice exposed to intense noise are summarized in Table I. Two noise levels were used to stimulate different animal groups. The first noise stimulus (114 db, 10–20 kc) may be likened in approximate magnitude to the noise produced next to a riveter but at a higher frequency. The other noise source approaches the levels produced by jet motors. It appears from Table I that neither of the noise stimuli caused any appreciable change in adrenal or plasma cholesterol levels. The mean adrenal and plasma cholesterol values of groups I and II differ from groups IV through VI since the first two groups were analyzed by the Sperry-Webb technique and the others by the method of Zlatkis.

Additional data included taking adrenal weights and measuring serum electrolytes at 0, 3, 6 h after noise exposure. The data in Table II reveal that noise likewise has no effect on adrenal weight or serum electrolyte concentrations. Although the failure to obtain adrenal hypertrophy or adrenal cholesterol depletion in the present study would seem to indicate that noise exposure was not followed by excessive adrenocortical activation, there always remains the possibility that chronic or continuous noise exposures could result in a breakdown of normal defense mechanism in the animal. Recently we conducted several studies of possible stress effects of prolonged exposure of mice, rats and guinea pigs to intense noise for periods ranging from one to four weeks duration. No evidence was obtained which would suggest an overactivation of the pituitary-adrenocortical system. This would seem to lend further credence to the assertion that homeostatic adjustment mechanisms of animals are not taxed to dangerous limits by intense sound stimulation.

One still left with the problem of explaining phenomena such as 'ultrasonic sickness' which have been described in men working around jet planes[6]. The major complaints associated with this disorder range from simple subjective expressions of weakness, fatigue and irritability to claims of impotence, infertility and loss of libido. Further studies are needed to evaluate the action of intense noise as a psycho-physiological stress since it has long been recognized that disturbances in reproductive capacity sometimes arise from anxiety situations without any observable evidence of functional impairments in the reproductive system.

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Department of Zoology and Entomology, Pennsylvania State University, University Park (Pennsylvania), October 30, 1957.

Résumé

Nos expériences sur l'effet du bruit intense sur l'activité des surrénales des souris ont donné les résultats suivants: 1° la cholestérine des surrénales et la cholestérine sanguine demeurent inchangées, 2° nous n'avons pas pu obtenir les signes d'hypertrophie de la surrénale ou d'atrophie du thymus après 1 à 4 semaines d'excitations par le bruit (5 min par jour). On constate donc que chez les souris périodiquement soumises à un bruit intense mais de courte durée, le cortex surrénalien ne subit pas d'augmentation excessive.

[5] A. Zlatkis, B. Zak, and A. Boyle, J. Lab. clin. Med. *41*, 486 (1953).

[6] P. Bugard, H. Souvras, P. Valade, E. Coste, and J. Salle, Sem. Hôp. Paris *29*, 1 (1953).

Table II

Adrenal weights and serum ion levels of mice exposed to intense noise (143 db, 150–4800 cy/s)

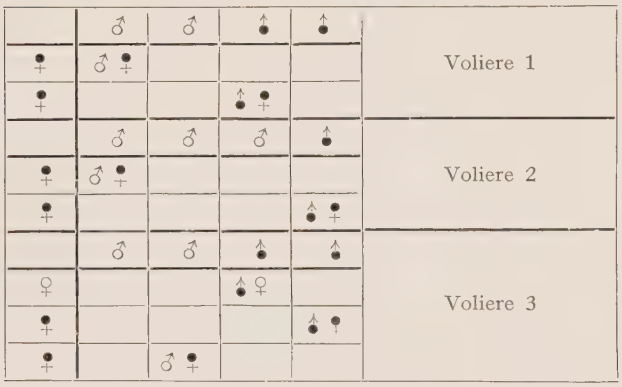
Animal	Sex	*n*	(g) Body weight	h after exposure	(mg) adrenal	Na	meq/l Ca	K
Control	M	4	29 ± 3	0	31 ± 4	160 ± 2	4·8 ± 0·5	4·6 ± 0·4
Experiment	M	4	28 ± 1	0	34 ± 6	161 ± 9	4·8 ± 0·4	4·5 ± 0·7
Control	F	4	30 ± 6	0	55 ± 4	154 ± 2	4·2 ± 0·4	4·4 ± 0·5
Experiment	F	4	27 ± 1	0	53 ± 6	159 ± 3	4·7 ± 0·2	4·2 ± 0·1
Control	M	4	30 ± 2	3	32 ± 2	158 ± 4	4·6 ± 0·5	5·3 ± 1·0
Experiment	M	4	31 ± 2	3	31 ± 2	151 ± 8	4·6 ± 0·2	4·2 ± 0·4
Control	F	4	28 ± 1	3	61 ± 7	161 ± 4	4·8 ± 0·5	4·5 ± 0·4
Experiment	F	4	24 ± 4	3	60 ± 11	159 ± 3	4·6 ± 0·2	4·5 ± 0·2
Control	M	4	28 ± 2	6	34 ± 6	153 ± 7	4·4 ± 0·1	3·8 ± 0·6
Experiment	M	4	29 ± 1	6	32 ± 7	148 ± 11	4·3 ± 0·7	3·5 ± 0·4
Control	F	4	23 ± 3	6	57 ± 6	162 ± 0	4·5 ± 0·6	4·7 ± 0·4
Experiment	F	4	26 ± 1	6	57 ± 6	162 ± 0	4·8 ± 0·2	4·9 ± 0·7

Soziale Verhaltensweisen bei hörenden und gehörlosen Dompfaffen (*Pyrrhula pyrrhula* L.)

Die sozialen Verhaltensweisen der Tiere werden von Schlüsselreizen gelenkt, die durch verschiedene Sinne wahrgenommen werden können. Wir haben uns gefragt, wie weit bei Tagvögeln die beiden wichtigsten Sinne, Gesicht und Gehör, bei der Verhaltenssteuerung beteiligt sind. Als Versuchstiere dienten Dompfaffen, deren Ethologie schon eingehend untersucht worden ist[1]. Einem Teil der Tiere wurde operativ die Cochlea entfernt, so dass sie völlig taub waren; auf das Allgemeinverhalten hat dieser Eingriff nach Abklingen der unmittelbaren Operationsfolgen keinen Einfluss[2]. Um die optischen Kontakte zwischen Gimpeln auszuschalten, wurden (hörende) Vögel in verdeckte Käfige gesetzt und anderen das Gefieder mit Methylenblau überfärbt. Die sozialen Gemeinschaften der Vögel in und ausserhalb der Brutzeit wurden in Freivolieren von etwa 10 m³ Inhalt beobachtet, deren Ausstattung dem natürlichen Biotop entsprach.

Tabelle I

Spontane Paarbildung von hörenden (♂, ♀) und gehörlosen (♂̣, ♀̣) Dompfaffen.



Verhaltensweisen während der Brutzeit. Beide Geschlechter des Dompfaffen singen in Volieren ebenso wie im Freien besonders in den Monaten vor und während der Paarbildung. Sechzehn gehörlose Vögel brachten noch ein, bzw. zwei Jahre nach der Ertaubung den – an sich klangarmen – Gesang unverändert und genau so häufig hervor wie hörende Vögel. Nur bei je einem ♂ und einem ♀ wandelte sich die Stimme mit der Zeit merklich ab.

Für die Anpaarung sind verschiedene Auslösesignale entwickelt[3]; das ♀ ist dabei der aktivere Teil, der zwischen verschiedenen ♂♂ wählt. In einem Teil unserer Versuche liessen wir die Paare sich frei aus kleineren Populationen von hörenden (♂, ♀) und gehörlosen (♂̣, ♀̣)* Vögeln kombinieren (Tab. I) und isolierten diese später. Eine Sonderstellung der tauben Gimpel bei der Paarbildung konnten wir nicht feststellen.

In den meisten zur Balz gehörenden Teilhandlungen wie «Schnabelflirt», «Zärtlichkeitsfüttern» und «Halmbalz» (Nicolai)[4] verhalten sich gehörlose Dompfaffen ebenso wie hörende nach Nicolai's und eigenen Erfahrungen. Auch konnten wir wiederholt beobachten, wie ein Partner eines Paares den anderen, ausserhalb der Voliere frei fliegenden, aus einiger Entfernung ohne akustische Hilfe individuell erkannte. Beim «Nestlocken» allerdings, das darin besteht, dass das ♂ dem ♀ mit auffälligen Bewegungen voranfliegt und es mit «bihr»-Rufen auf den von ihm gewählten Nistplatz zu leiten sucht, stellten wir bei gehörlosen Vögeln in zwei Fällen Fehlleistungen fest. Das ♀̣ baute an anderer Stelle, während das ♂ weiter am erstgewählten Ort lockte.

Die Begattung wird durch das ♀ eingeleitet, das sich hinduckt und leise «dididi» ruft[4]. Gehörlose ♂̣♂̣ kamen in einigen Fällen dieser Aufforderung nicht nach; leider fehlen uns ausreichende Kontrollbeobachtungen an hörenden ♂♂. Die Begattung verlief bei allen gehörlosen Dompfaffen sonst störungsfrei. Fünf ♀̣♀̣ legten insgesamt 29 Eier, die alle bis auf vier befruchtet waren. Gerade die unbefruchteten Eier stammten von gemischten Paaren mit normalen ♂♂.

Die Bindung an Nest und Gelege ist gegen Ende der Bebrütung so fest, dass die Vögel in die geöffnete Voliere freiwillig zurückkehren; dies ausnutzend konnten wir optimale Nahrungszusammensetzung für die frisch geschlüpften Jungvögel sichern. Hörende Altvögel brachten bei uns fünf Gelege bis zum Flüggewerden hoch. Dabei haben auch wir die von Nicolai beschriebenen «didi»-Laute (bis zum vierten Tag) und Sperrlaute (anschliessend) vernommen. Alle unsere Paare (sieben) mit einem oder zwei gehörlosen Partnern versagten jedoch bei der Jungenaufzucht. Nur in einem Fall (♂♀̣) kamen zwei Junge hoch; als das ♀̣ (!) in der Versorgung nachliess und sich am 12. Tag nicht mehr um die Brut kümmerte, fütterte das ♂ allein weiter. Die schon in der Entwicklung zurückgebliebenen Jungen wurden nur dadurch am Leben erhalten, dass wir sie nachts in einen geheizten Raum verbrachten. Das Hudern gehört nicht zum Verhaltensinventar des ♂. Die übrigen Paare versorgten ihre Jungen unzureichend, so dass diese im Mittel nicht über fünf Tage alt wurden. Ausserdem waren bei den Altvögeln die dem Geschlechtspartner zugewandten, den Zusammenhalt des Paares sichernden Instinkthandlungen gestört oder ganz erloschen.

Verhaltensweisen ausserhalb der Fortpflanzungszeit. Etwa vom September an bis in den Februar leben die Dompfaffen gesellig in lockeren Schwärmen. Der Lockruf ist das wichtigste Signal für das Zusammenfinden des winterlichen Trupps. Ein versprengter Vogel lockt regelmässig mit dem bekannten flötenden Ruf, der von anderen Dompfaffen beantwortet wird. Ein isolierter Gimpel in einem Fangkäfig übt durch sein Locken eine starke Anziehung auf andere Artgenossen aus. Die leuchtend rote Färbung des ♂ spielt dabei keine Rolle. Zweieinhalb Monate hindurch wechselten wir an einem Fangplatz täglich zwischen ♂ und ♀ als Lockvogel. In dieser Zeit kirrte das ♂ 17, das unscheinbare ♀ 16 Wildlinge. In einer anderen Versuchsreihe änderte auch das völlige Überfärben der Lockvögel (♂ und ♀) mit Methylenblau nicht deren Lockkraft. Das Geschlechtsverhältnis der Fänge wird nicht von den (unverdeckten) Geschlechtsmerkmalen des Lockvogels bestimmt; es schwankt je nach der Jahreszeit zwischen 1,2 ♂ und 2,5 ♂ : 1 ♀.

[1] J. Schwartzkopff, Z. vgl. Physiol. *31*, 527 (1949). – G. Kramer und U. v. St. Paul, Behaviour *3*, 243 (1951). – J. Nicolai, Z. Tierpsychol. *13*, 93 (1956).

[2] J. Schwartzkopff, Z. vgl. Physiol. *31*, 527 (1949).

[3] J. Schwartzkopff, Z. vgl. Physiol. *31*, 527 (1949). – J. Nicolai, Z. Tierpsychol. *13*, 93 (1956).

* Aus technischen Gründen wurde der konventionelle Schrägpfeil senkrecht gesetzt.

In der Häufigkeit des spontanen Lockens unterscheiden sich taube Vögel nicht von hörenden; sowie allerdings ein lockender Dompfaff aus der Ferne Antwort vernimmt, steigt seine Ruffreudigkeit. Wir verhörten von Artgenossen akustisch isolierte Dompfaffen am frühen Morgen nach dem Erwachen. Wie Tabelle II zeigt, ist die spontane Lautproduktion zwischen Hörenden und Gehörlosen praktisch gleich.

Tabelle II

Spontane Lautäusserungen (Rufe/h) von hörenden (5 Vt.) und tauben (6 Vt.) Dompfaffen

	Hörend	Taub
Lockruf	5,8	5,9
Stimmfühlungslaut	17	13

Innerhalb des sozialen Verbandes stellt sich bei Gimpeln eine lineare Rangordnung ein, wie gelegentliche Freilandbeobachtungen und die Untersuchung der Vögel in den Volieren zeigten. Mit Drohlauten und -gesten[4] verteidigt ein Spitzenvogel seinen Platz am Futternapf oder auf der Sitzstange. Dabei stehen die ♀♀ in der Hackordnung über den ♂♂. Schon früher stellte Schwartzkopff fest[2], dass gehörlose Dompfaffen in der Rangordnung keine Sonderstellung einnehmen. Dies wird an den jetzt untersuchten Vögeln bestätigt (Tab. III).

Tabelle III

Hackordnungen in verschiedenen Trupps (Volieren-Populationen) von hörenden (♂, ♀) und gehörlosen (♂̸, ♀̸) Dompfaffen.

Voliere:	1	2	3	4	5
1.	♂	♂	♂̸	♀̸	♀̸
2.	♀̸	♀̸	♀	♀	♀
3.	♂̸	♂	♂	♀	♀̸
4.	♂				♀̸

Interessanterweise kam es zweimal zu heftigen Rangordnungskämpfen, als überfärbte ♀♀ einem Trupp zugesetzt wurden; auch die sonst das Feld räumenden ♂♂ bekämpften die nicht erkennbaren ♀♀ zunächst. Im Endergebnis setzten diese sich aber durch. Hier ist ein deutlicher Hinweis dafür, dass die Einordnung in den sozialen Verband nicht nur durch die von den einzelnen Gliedern ausgehenden Signale, sondern auch durch die «eigene Meinung» eines Individuums über seine Stellung bestimmt wird.

Besondere Mühe haben wir darauf verwandt, die stimmungsübertragende Wirkung von akustischen und optischen Warn- und Schrecksignalen zu prüfen. Gelegenheitsbeobachtungen aus komplexen Situationen liessen sich unter kontrollierten Bedingungen nicht reproduzieren. Auch Kramer und v. St. Paul[5] hatten Schwierigkeiten, Schreckreaktionen von Gimpeln zu erzielen. Es scheint, dass mit Sicherheit ein Schwarm nur durch den Schreckstart eines Artgenossen (optisch) in die Flucht gerissen wird. Akustische Signale allein hatten in der Mehrzahl unserer Versuche keine nachweisbare Wirkung.

Diskussion. Trotz Angleichung unserer Untersuchungsbedingungen an die natürlichen Lebensverhältnisse des Dompfaffen werden sich nicht alle Zweifel hinsichtlich der Verallgemeinerung der Befunde ausräumen lassen. Wenn zum Beispiel auch gehörlose Vögel von der Balz bis zum Ausbrüten der Jungen die arttypischen Instinkthandlungen ohne wesentliche Störungen vollziehen, so können hier ebensowohl die räumliche Umgrenzung durch die Voliere, die der Paarbildung günstig ist, wie «doppelte Sicherung» durch angeborene auslösende Mechanismen[6] über verschiedene Sinne mitspielen. Sicher ist, dass die Bereitschaft der Eltern, ihre Brut zu hudern und zu füttern, erlischt, wenn die betreffenden Instinkthandlungen nicht durch akustische Signale von den Jungen ständig neu ausgelöst werden. Zur Zeit der Schwarmbildung wird ein versprengter Vogel rein akustisch angelockt. Die Produktion der arteigenen Laute bedarf aber nicht der Kontrolle durch das Gehör. Soziale Funktionen des Schwarms wie Rangordnung und Alarmierung werden überwiegend optisch gesteuert.

R. Hüchtker und J. Schwartzkopff

Zoologisches Institut der Universität Göttingen, 24. Oktober 1957.

Summary

The behaviour of bullfinches (Pyrrhula) after removal of the cochlea is studied. The influence of acoustical and optical stimuli upon the social contacts is compared. Parts of the social behaviour are controlled mainly acoustically, e.g. the attraction of a single bird by a calling fellow. Also the final raising of young birds is not possible without acoustical contacts between parents and offspring. The formation of pairs, however, nest-building, laying of fertilized eggs, and breeding are performed normally by birds without hearing. Also the social order within a group of bullfinches regulates itself without acoustical perception by its members. The alarming behaviour seems to be transferred within a group by optical signals.

[4] J. Nicolai, Z. Tierpsychol. *5*, 235 (1943).

[5] G. Kramer und U. v. St. Paul, Behaviour *3*, 243 (1951).

[6] K. Lorenz, Z. Tierpsychol. *5*, 235 (1943).

Psilocybin, ein psychotroper Wirkstoff aus dem mexikanischen Rauschpilz *Psilocybe mexicana* Heim

Die Geschichte der mexikanischen Rauschpilze, welche von den eingeborenen Indianern schon in vorkolumbanischer Zeit bei rituellen Zeremonien und von Wahrsagern zur Erlangung hellseherischer Fähigkeiten eingenommen wurden und auch heute noch zu den gleichen Zwecken verwendet werden, bildet Gegenstand einer früheren Publikation des einen von uns[1]. Das Ehepaar Wasson hat auf mehreren Expeditionen in die abgelegenen Gebiete Mexikos in den Jahren 1953 bis 1955 den heutigen Gebrauch dieser Pilze studiert und die bei solchen Zeremonien miterlebten halluzinatorischen Zustände eingehend beschrieben[2]. Verschiedene damals gesammelte Pilze, sowie reichliches Pilzmaterial, das der eine von uns anlässlich einer Expedition im Juli/August 1956 zusammen mit R. G. Wasson in die Territorien der Mazateken, Chatinos und Azteken gesammelt hat, erlaubten die Bestimmung und Charakterisierung der wichtigsten, meist neuen Arten dieser Rauschpilze, welche den Gattungen *Psilocybe*

[1] R. Heim, C. R. Acad. Sci. *242*, 965 (1956).

[2] Valentina P. Wasson und R. G. Wasson, *Mushrooms, Russia and History* (Pantheon Books, New York 1957).

(mindestens 6 Arten), *Stropharia* (1 Art) und *Conocybe* (1 Art) angehören[3].

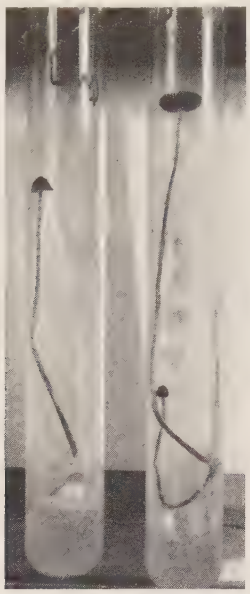


Abb. 1. *Psilocybe mexicana* Heim, Fruchtkörper auf Malzagar (Phot. A. B.)

Es gelang, von einigen dieser Pilzarten im Laboratorium Kulturen anzulegen. Von einer Spezies, nämlich von *Psilocybe mexicana* Heim (siehe Abb. 1), liessen sich grössere Mengen wirksamer Fruchtkörper züchten[4]. Ferner konnten Kulturbedingungen gefunden werden, unter denen diese Spezies *in vitro* Sklerotien bildet, die, wie Selbstversuche zeigten, gleichfalls psychotrop wirksam waren. Die Gewinnung von Sklerotien, welche rationeller zu grösseren Mengen von aktivem Ausgangsmaterial führt, wird andernorts eingehender beschrieben[5].

Sowohl aus den Fruchtkörpern, als auch aus den Sklerotien von *Psilocybe mexicana* Heim liess sich das gleiche wirksame Prinzip auf die nachstehend beschriebene Weise isolieren, wobei alle Fraktionen durch Selbstversuche getestet wurden.

Der Methanolextrakt des sorgfältig getrockneten, feinstpulverisierten Pilzmaterials wurde zur Entfernung von unwirksamen Begleitstoffen nacheinander mit Petroläther, Chloroform und Chloroform/Alkohol behandelt. Aus dem verbleibenden Rückstand trennte man weitere Ballaststoffe durch Lösen in wenig Wasser und Fällen mit absolutem Alkohol ab. Der Eindampfrückstand des Filtrates wurde an einer Zellulosesäule unter Verwendung von wassergesättigtem Butanol nach dem Durchlaufverfahren chromatographiert. Nach einem dunkelgefärbten, inaktiven Vorlauf erschienen Fraktionen, die mit Keller-Reagens (eisenchloridhaltiger Eisessig + konzentrierte Schwefelsäure) eine blaue bis violette Farbreaktion gaben. Diese wurden vereinigt und erneut einer sorgfältigen chromatographischen Analyse an Zellulosepulver mit wassergesättigtem Butanol unterworfen. Dabei liessen sich zwei getrennte Zonen mit positiver Keller-Reaktion unterscheiden, eine rascher wandernde, mengenmässig geringe Zone, die eine blaue Färbung gab, sowie eine zweite, durch eine violette Farbreaktion gekennzeichnete Hauptzone.

Die Hauptzone lieferte ein amorphes, in Wasser spielend lösliches, noch halogenhaltiges, hochaktives Pulver. Nach der Behandlung der wässerigen Lösung mit Silberkarbonat und Entsilbern des Filtrates mit Schwefelwasserstoff kristallisierte der Wirkstoff, den wir *Psilocybin* genannt haben, aus der konzentrierten wässerigen Lösung in feinen weissen Nadeln. Ausbeute: zum Beispiel aus getrockneten Fruchtkörpern 0,4%. Nach einmaligem Umkristallisieren aus Wasser, in dem sich der Wirkstoff in 20 Teilen bei Siedehitze löst, oder aus 120 Teilen heissem Methanol, aus dem sich das *Psilocybin* in farblosen Prismen abscheidet, ist die Verbindung analysenrein. In organischen Lösungsmitteln wie Aethanol, Chloroform oder Benzol ist *Psilocybin* praktisch unlöslich. Der Wirkstoff besitzt amphoteren Charakter. Die wässerig-alkoholische Lösung zeigt ein pH = 5,2. In wässerigen Säuren oder Laugen ist *Psilocybin* spielend löslich. In der evakuierten Kapillare schmilzt die HV.-trockene Substanz unscharf zwischen 185–195° (korr.) $\alpha_D^{20} = 0°$ ($\pm$ 0,02°) (c = 0,5 in 50prozentigem Methanol, 2-dm-Rohr). Für die Analyse wurde im Hochvakuum bei 100° getrocknet, wobei das Wasser-Kristallisat 25,4%, das Methanol-Kristallisat 10,4% Gewichtsverlust zeigten.

Die Elementaranalyse lieferte folgende Werte: C 49,94; 49,98%. H 6,10; 6,13%. O 16,33%, N 8,65; 8,91%, P 19,68; 19,83%. Diese Analysenzahlen stimmen auf eine Bruttoformel $C_{13}H_{18'20'}O_3N_2P_2$.

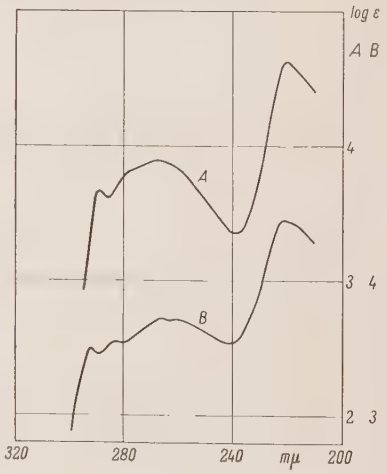


Abb. 2.
A: Psylocybin } in Methanol
B: Psilocin }

Das UV-Spektrum (Abb. 2*A*) zeigt Maxima bei 222, 267 und 290 mμ, und besitzt den Charakter eines im Benzolring substituierten Indols. Für den Indolcharakter spricht auch die violette Kellersche Farbreaktion. Im *Psilocybin* scheint somit ein neuartiges, phosphorhaltiges Indolderivat vorzuliegen. Im IR-Spektrum (Abb. 3) fällt vor allem eine Bande bei 2350 cm^{-1} auf, die einer P-H-Schwingung zugeordnet werden könnte.

Aus der rascher wandernden Chromatogrammzone mit positiver Keller-Reaktion liess sich in sehr geringer Ausbeute eine Substanz gewinnen, die zum Unterschied vom *Psilocybin* mit eisenchloridhaltigem Eisessig und konzentriert Schwefelsäure eine rein blaue Färbung gibt, und die wir *Psilocin* genannt haben. *Psilocin* unterscheidet sich auch im UV-Spektrum mit Maxima bei 222, 260, 267, 283 und 293 mμ (Abb. 2, *B*) deutlich vom *Psilocybin*. Da

[3] R. Heim, C. R. Acad. Sci. *242*, 1389 (1956); *244*, 695 (1957); Revue de Mycologie *22*, 20, 36 (1957).

[4] R. Heim und R. Cailleux, C. R. Acad. Sci. *244*, 3109 (1957); *245*, 597, 1761 (1957).

[5] R. Heim, A. Brack, H. Kobel, A. Hofmann und R. Cailleux, C. R. Acad. Sci. *247* (3. März 1958, im Druck).

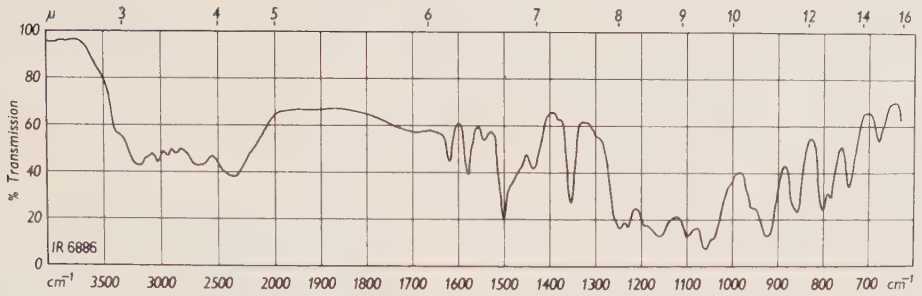


Abb. 3. IR.-Spektrum von Psilocybin in KBr.

Psilocin äusserst zersetzlich ist, konnte es bis jetzt nicht zur Analyse gebracht werden.

Psilocybin zeigt bei peroraler Applikation beim Menschen die gleiche psychotrope Wirkung wie der Pilz. Nach der Einnahme von 4 bis 8 mg *Psilocybin* entsteht nach etwa $^3/_4$ h ein mehrere Stunden andauernder Rauschzustand mit körperlicher Entspannung und ausgesprochenen psychischen Alterationen, der keinerlei Nachwirkungen hinterlässt. Die Symptome variieren individuell und sind zum Teil ähnlich den durch Mezkalin und Lysergsäurediäthylamid (LSD) erzeugten Wirkungen. Damit ist es zum ersten Mal gelungen, aus einem mexikanischen Rauschpilz das wirksame Prinzip in kristallisierter Form zu isolieren und chemisch zu charakterisieren.

Die Analysen wurden im mikroanalytischen Laboratorium SANDOZ (Dr. W. Schöniger) ausgeführt, die Spektren in der spektralanalytischen Abteilung (Dr. H. G. Leemann) aufgenommen. Wir danken Herrn H. Tscherter für sehr geschickte, experimentelle Mitarbeit.

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und H. Kobel

Pharmazeutisch-chemisches Laboratorium SANDOZ, Basel, und Laboratoire de Cryptogamie du Muséum National d'Histoire Naturelle, Paris, 17. Februar 1958.

Summary

The active principle of *Psilocybe mexicana* Heim, a mexican mushroom with hallucinogenic properties, has been isolated in crystalline form. The compound has been given the name *Psilocybin;* it possesses indole characteristics and contains phosphorus. A second substance, closely related to *Psilocybin* but found only in traces, has been called *Psilocin.*

PRO EXPERIMENTIS

Solutions of Potato-Root Diffusate of Low Ion Content

There are two reasons why it is desirable to obtain solutions of root diffusates of very low ionic content: both arise from the demonstration that the hatching factor of the potato-root eelworm (*Heterodera rostochiensis* Wollenweber) possesses cardiotonic properties and can be assayed by means of a frog heart preparation[1]. Firstly, for the heart test, the ionic composition of the perfusion fluid must be accurately controlled. Secondly, recent work has shown that cardiac glycosides may influence sodium-potassium transport[2]: if the potato-root eelworm hatching factor works in a similar way, the hatching process should also be sensitive to the ionic background. This we have found to be the case[3]; as will be shown in full elsewhere, hatching can be increased by $CaCl_2$ and, although the position is here not quite so clear, decreased by KCl. The standardisation of solutions by the hatching test or by the heart assay can therefore be completely misleading unless the ionic composition is controlled.

Solutions of diffusate containing the potato-root eelworm hatching factor are usually obtained from potato or tomato plants grown in pots; these are 'leached' at varying times with known volumes of water, the same plants being used repeatedly during the period of their active growth. Some workers put the leachings back through the pots twice more in an endeavour to obtain a stronger solution. Since the plants are raised in a medium containing minerals, and since tap water is usually used, it is clear that the solutions will contain appreciable and variable quantities of salts. We have found, leaching with 100 ml lots of distilled water, that the conductivity of the leachings was equivalent to 0·14% NaCl; and this rose approximately 20% if the leachings were put through the pots twice more. Moreover, these leachings were from plants grown in John Innes seed compost from which the minerals were omitted.

In this laboratory, root diffusates have always been obtained directly from the roots[4]. The plants are lifted, their roots thoroughly washed, and the whole root system then stood in water, usually for 2 h; originally tap water was used, later distilled water. It has always been felt that this method would give a purer product, but, until recently it was not realised that by this method solutions could be obtained virtually ion free. The method has also been used successfully with hosts of other species of *Heterodera.*

In a recent test, the roots of four fully grown plants were thoroughly washed, first in tap water, finally in distilled water. They were immersed, tops still attached, in a litre of ion exchange water equal to triple glass distilled, and placed in a polythene bucket, in a greenhouse. Samples removed at intervals during a 24 h period showed little change from a very low conductivity, the value being equivalent to 0·012% NaCl at the outset,

[1] C. Ellenby and A. B. Gilbert, Nature *180*, 1105 (1957).

[2] H. J. Schatzmann, Helv. physiol. Acta *11*, 346 (1953). – M. Weatherall and B. Rayner, Personal communication. – V. K. Johnsen, Personal communication. – J. B. Kahn Jr. and G. H. Acheson, J. Pharmacol. *115*, 305 (1955).

[3] C. Ellenby and A. B. Gilbert, Unpublished.

[4] C. Ellenby, Nature *154*, 363 (1944).

and 0·017% after 24 h; a parallel test gave values of 0·015 and 0·018 respectively. Our best performance to date gave a value after 24 h equivalent to only 0·01% NaCl but we feel that even this can be improved, for, since the plants were raised in the compost mentioned above, we were operating under unfavourable conditions; the peat granules adhere very intimately to the roots and, even after prolonged washing, not all particles are removed. In future we intend raising the plants in vermiculite.

After 24 h, the plants still stand well. Indeed, we have kept plants in this fashion for some days, but there appears to be a decrease in the output of the hatching factor after this period and we have never used such solutions.

For small volumes of solution, filtration through filter paper should be avoided if possible; in a test with 20 ml samples, we found, in some cases, that if all the increase in conductivity were due to salts, this was more than doubled by passage through a Whatman No. 40 acid extracted filter paper. The increases appear to be connected with the nature of the solutions for they were much less with distilled water, our samples of Whatman No. 1 being superior in this respect to the No. 40. The total increase is slight where large volumes of solutions are dealt with; solutions are then filtered through a Büchner funnel after giving the filter paper a preliminary wash with at least 100 ml of high grade distilled water. Resistance glass and polythene bottles are used throughout.

Although the solutions are of very low ionic content, our investigations have shown that, if it were all calcium chloride, its addition to low calcium Ringer would be sufficient, very occasionally, to increase the beat of the frog heart even in the absence of the hatching factor, since the heart is very sensitive to Ca^{++}; the assay would therefore be misleading. However, the 24-h solutions are very strong; they can therefore be diluted until it is quite certain that their ionic contribution to the low calcium Ringer in which they are assayed can safely be ignored. Such an assay showed that the initial solution obtained from the four plants after 24 h was about 10 times as strong as the minimal concentration of Cambridge 'standard solid'[5] hatching factor; the yield from the four plants in 24 h was therefore equivalent to more than 1 g of the Cambridge solid. We have since found that even stronger solutions can be obtained from the small root systems of younger plants; these can be contained in smaller quantities of water.

This work forms part of a programme supported by a grant from the Nuffield Foundation to whom we have pleasure in expressing our thanks.

C. Ellenby and A. B. Gilbert

Department of Zoology, University of Durham, Newcastle upon Tyne, October 14, 1957.

Zusammenfassung

Zur quantitativen Bestimmung des Schlüpffaktors der Kartoffelnematode, entweder mit sogenanntem Schlüpftest, oder durch unsere, vor kurzem beschriebene Froschherzmethode, muss die Ionenkonzentration in der Lösung kontrolliert werden. Eine Methode zur Herstellung geeigneter Lösungen wird beschrieben.

[5] C. T. Calam, H. Raistrick, and A. R. Todd, Biochem. J. *45*, 513 (1949).

Obtention de lignées cellulaires en culture de tissus d'invertébrés

Le rôle prépondérant des cultures de tissus dans la recherche médicale, notamment en virologie, est avant tout le résultat de l'obtention de souches de cellules à partir de tissus humains ou de vertébrés, avec possibilité de maintien de celles-ci.

Chez les invertébrés, plusieurs tentatives de cultures de différents tissus ont été faites soit pour l'étude physiologique, soit en vue de travaux de pathologie cellulaire. Dans quelques cas on a noté, à partir d'un explant, l'émigration de fibroblastes ou de l'épithélium; ces développements étaient toujours réduits[1] et ce n'est que rarement que l'on a observé des mitoses ou des divisions amitotiques[2]. Mais les repiquages successifs des cultures en voie de multiplication et la formation de souches cellulaires, critères actuels des cultures de tissus, n'ont pas été réalisés.

Nos expériences ont porté parallèlement sur deux lépidoptères: *Bombyx mori* L., et *Galleria mellonella* L., sur un orthoptère *Blabera fusca* et sur les mollusques: *Helix aspersa* Muller et *H. pomatia* L. Nous résumons ici ce qui concerne la culture des lignées relatives à *B. mori* et *H. aspersa* les plus anciennement maintenues.

L'enceinte de culture a été choisie de telle manière qu'on associe les procédés de multiplication modernes sur grande surface avec changement de milieu et les repiquages aseptiques. Nous avons abandonné les techniques en goutte pendante, sur lame détachée et sur anneaux, ainsi que les procédés septiques, pratiquement les seuls à être employés jusqu'alors pour les insectes ou pour les mollusques. Les cultures ont été réalisées en tubes roulants, en tubes plats avec lame intérieure en boîtes de Kolle et de Roux et dans des flacons permettant le contrôle au microscope, à l'immersion sans interruption de la culture[3].

Les tissus de *B. mori* ont été cultivés dans un milieu contenant pour 1000 cm^3 d'eau bi-distillée, les éléments minéraux suivants: KCl: 3,0 g; $CaCl_2$: 0,5 g; NaH_2PO_4: 1,0 g; Mg $SO_4 7H_2O$: 3,5 g; $MgCl_2 6H_2O$: 3,0 g; CO_3NaH jusqu'à pH 6,4. Les glucides étaient représentés par le glucose à raison de 1 g °/°° et la source d'acides aminés par l'hydrolysat de caséine (Vitrum) ayant montré préalablement une certaine polyvalence pour les tissus de vertébrés. Ce produit (0,5 g) a été complété de glutamine (0,1 °/°°). Les facteurs de croissance étant peu connus chez les insectes, un extrait de levure (obtenu par effet de NaCl) à raison de 2 cm^3 °/°°, avec choline à 0,002 °/°° a été ajouté. En plus de 200000 U.I. °/°° de pénicilline et de 50 mg °/°° de streptomycine le milieu est additionné d'hémolymphe de *B. mori* (10%) chauffée à 60°C pendant 5 min pour éliminer l'effet de la tyrosinase[4].

A partir des explants de gonades femelles de larves à la fin du 5e âge, l'émigration des cellules type «fibroblastes» s'intensifie à 30°C, après 48 h, avec formation

[1] W. E. Beckel, Nature *177*, 534 (1956). – P. Bohuslav, Arch. exp. Zellforsch. *14*, 1 (1933). – J. G. H. Frew, Brit. J. exp. Biol. *6*, 1 (1928). – J. B. Gatenby, J. C. Hill et T. J. Macdougald, Quart. J. micr. Sci. *77*, 129 (1935). – A. J. P. Goodchild, Nature *173*, 504 (1954). – T. D. C. Grace, Nature *174*, 187 (1954). – M. R. Murray, Proc. Soc. exp. Biol., N.Y. *6*, 1 (1928).

[2] J. B. Gatenby, J. C. Hill et T. J. Macdougald, Quart. J. micr. Sci. *77*, 129 (1935). – W. Trager, J. exp. Med. *61*, 501 (1935). – S. S. Wyatt, J. gen. Physiol. *39*, 941 (1956).

[3] C. Vago, Mikroskopie (sous presse).

[4] S. S. Wyatt, J. gen. Physiol. *39*, 941 (1956).

d'un réseau. Le halo prélevé tous les 8–14 jours et transposé sur de nombreux points dans un autre flacon de culture, assure la continuation d'un développement lent des fibroblastes avec formation d'un tissu à structure moyennement serrée. Dans les subcultures apparaissent quelquefois aussi des formes épithéliales. Certaines lignées ont été repiquées douze fois (Figure).

Culture de tissu de gonade femelle de larve de *Bombyx mori*. Culture de 5 jours, troisième transfert. Hémalun-éosine-orange 1:600.

Pour les tissus du mollusque *H. aspersa*, le milieu contenait pour 1000 cm³ d'eau bi-distillée: NaCl: 6,500 g; KCl: 0,14 g; $CaCl_2$: 0,12 g; NaH_2PO_4: 0,01 g; CO_3NaH: 0,20 g; glucose: 1,0 g; avec hydrolysat de caséine, glutamine, extrait de levure, choline et antibiotiques aux mêmes doses que pour *B. mori*. Le pH du sang augmente très rapidement après prélèvement. Aussi est-il immédiatement incorporé dans le milieu (10%) et centrifugé ensemble afin d'éliminer les hémocytes et obtenir un pH définitif de 7,6–7,9.

Les explants de la tunique pédieuse (non septique!) donnent naissance aux fibroblastes, lesquels prélevés et repiqués, forment des réseaux à évolution très lente. Après huit repiquages, les cellules se maintiennent avec peu de granules et un nombre réduit de mitoses.

Ces résultats montrent la possibilité de maintenir à partir des tissus d'invertébrés, des lignées cellulaires ininterrompues et de rendre accessibles ces tissus aux travaux de pathologie expérimentale *in vitro*. Nous soulignons cependant, que le développement des cellules après repiquages est beaucoup plus lent que celui des tissus de vertébrés en général. Nous attribuons ce fait à l'insuffisance de nos connaissances relatives aux facteurs de croissance chez les insectes et chez les mollusques. Cette question est d'ailleurs encore actuellement complexe pour les cultures de tissus de vertébrés. Les extraits obtenus à partir de l'organe de Bojanus et des ovaires des *Helix* semblent constituer un facteur accélérateur et c'est par l'adjonction de ce principe que nous essayons à l'heure actuelle d'augmenter la croissance. D'autre part, la blessure de la culture semble libérer, comme cela a été signalé pour la culture de tissus de vertébrés, certaines substances activatrices. Enfin, pour assurer une homogénéité de développement, nous continuons à l'heure actuelle l'isolement des lignées à l'aide de dispersions cellulaires par voie enzymatique.

C. Vago et S. Chastang

Institut National de la Recherche Agronomique, Station de Recherches d'Alès (France) (Laboratoire de Pathologie Comparée), le 10 décembre 1957.

Zusammenfassung

In Gewebekulturen von *Bombyx mori* ♀-Gonade und und von *Helix aspersa*-Bindegewebe abimpfbare Fibroblasten-Zellenstämme wurden erhalten. Die Zusammensetzung der Nährflüssigkeiten ist gegeben, und es wird auf Schwierigkeiten in der Gewebekultur Wirbelloser hingewiesen.

Culture of Nucellar Tissue of *Citrus in vitro*

A variety of reproductive structures in plants, such as anthers, ovules, endosperm, embryos and embryo fragments, have been successfully cultured *in vitro*; but I have not come across any report of the culture of nucellar tissue. In *Citrus*, the cells of nucellus surrounding the micropylar part of the embryo sac are known to produce adventive embryos. It was, therefore, considered worthwhile to make *in vitro* cultures of the nucellus in order to explore its proliferative capacity. A horticultural variety of *Citrus*, growing in the 'Varsity Campus, was used for experimentation.

The upper half of the nucellus, in which proembryos had been initiated, was dissected out and inoculated on White's[1] medium modified by the addition of Nitsch's[2] trace elements and cobalt chloride (0·025 ppm).

On this basal medium, the nucellus turned brown within a week. But in a medium supplemented with 400 ppm of casein hydrolysate (acid hydrolysed, neutralised and vitamin-free), profuse proliferation of nucellar cells was observed and the original tissue could hardly be traced as such 6–8 weeks after culture. Instead, there were produced abundant masses of tissue which are here designated as 'pseudobulbils' (Fig. 1) – a term first coined by La Rue[3] to designate the curious balls of tissue obtained in cultures of the female gametophyte of *Zamia*.

The pseudobulbils were pearly white and consisted of parenchymatous cells. They have now been maintained for over a year through several transfers to fresh media. Kinetin (2 ppm), added in conjunction with casein hydrolysate (400 ppm) to the basal medium, was found to enhance the production of pseudobulbils. It is possible that, apart from the proliferation of nucellar cells, the pseudobulbils may in part also originate from callusing of proembryos.

Pseudobulbils, left in culture media containing casein hydrolysate alone (400 ppm), and in combination with kinetin (2 ppm), for more than two months, occasionally developed into seedlings which presented several

[1] P. R. White, *A Handbook of Plant Tissue Culture* (Lancaster, Pa. James Cattell, 1943).

[2] J. P. Nitsch, Amer. J. Bot. *38*, 566 (1951).

[3] C. D. La Rue, Brookhaven, nat. Lab. Symp. No. 6, 187 (1954).

Fig. 1. – Pseudobulbils; 6 week-old on White's medium + casein hydrolysate. × 1·4.

Fig. 2. – Seedlings with normal leaves and fasciated hypocotyl. × 1·4.

morphological peculiarities like pluricotyly and the fasciation of cotyledons, stem and hypocotyl. The leaves, however, were normal (Fig. 2) and characteristic of the plant. They were green, gland-dotted, narrow-winged and articulated.

Proembryos, whether zygotic or nucellar, excised and cultured on the casein hydrolysate medium, showed normal development and attained the heart-shaped stage in 6–8 weeks of culture. Embryos at the torpedo stage on the same medium showed the plumule to have developed as a green papilla, which lay dormant even after transfers to fresh medium. No growth of the embryos was observed on the control medium.

The production of pseudobulbils from the nucellus opens new avenues of obtaining vigorous plants breeding true to the maternal parent. It may, however, be added that 'although a method of inducing adventive embryony has undoubted possibilities, there is also a need sometimes for the elimination of adventive embryos'[4].

[4] P. Maheshwari, *An Introduction to the Embryology of Angiosperms* (McGraw-Hill, N.Y. 1950).

It would be of great interest if some control could be exercised through chemical means to eliminate either zygotic or nucellar embryos to suit the needs of the breeder.

I am grateful to Professor P. Maheshwari and Dr. S. Narayanaswami for guidance, and to Professor Kenneth V. Thimann (Harvard University) for valuable suggestions. Grateful acknowledgements are made to the Council of Scientific and Industrial Research, New Delhi, for financing the scheme under which this work was carried out.

N. S. Ranga Swamy

Department of Botany, University of Delhi (India), October 10, 1957.

Zusammenfassung

Wucherungen, die in der vorliegenden Arbeit als «Pseudobulbillen» bezeichnet worden sind, erschienen zuerst in Kulturen von *Citrus*-Nucellargewebe auf einem künstlichen Nährboden. Diese Bulbillen sind durch zahlreiche Passagen in einem genau definierten Nährboden seit einem Jahr erhalten geblieben.

Nouveaux livres - Recensioni - Buchbesprechungen - Reviews

Physikalisches Wörterbuch

Herausgegeben von W. H. Westphal
VI, 833 und 795 Seiten. 4°
Zwei Teile in einem Band
(Springer-Verlag, Berlin, Göttingen und Heidelberg, 1952). DM 148.–

Dieses überaus umfangreiche Werk mit mehr als 10000 Stichworten und mit über 1500 Figuren im Text ist als Fortführung der 2. Auflage des Physikalischen Handwörterbuches von A. Berliner und K. Scheel (1932) gedacht. Das Wörterbuch wendet sich zwar, wie es im Vorwort heisst, in erster Linie an Physiker. Es soll aber auch Chemikern, Biologen und Medizinern dienlich sein. Nach Auffassung des Referenten kommt ein derartiges Werk vor allem für solche Interessenten in Frage, die dem behandelten Fachgebiet etwas ferner stehen, und die sich rasch über das eine oder andere Thema informieren wollen. Deshalb erscheint es berechtigt, dass das Buch durch einen Nichtphysiker besprochen wird.

Ohne Zweifel war es eine sehr grosse Mühe, die Beiträge der 80 Mitarbeiter aufeinander abzustimmen und dabei auch das wirklich Wesentliche hervorheben zu lassen. Vieles oder sehr wahrscheinlich das meiste aus dem heute so weitschichtigen Gebiet der Physik und aller angrenzenden Wissenschaften ist sehr klar dargelegt. Einige Artikel bestechen durch ihre hervorragende Prägnanz. Dem Referenten fällt es indessen auf, dass manches vielleicht zu ausführlich gegenüber anderen, heute wohl aktuelleren Dingen behandelt worden ist (Beispiel: Foucaultscher Pendelversuch). Es wundert den Referenten auch, dass gelegentlich Literatur zitiert worden ist, die heute eher als etwas überholt angesehen werden darf. Der Leser wird neueste Monographien oder zusammenfassende Darstellungen suchen. (Beispiel: Unter «Magnetochemie» ist das Buch von P. W. Selwood «*Magnetochemistry*» [1943] nicht erwähnt worden.) Der Referent ist weiterhin beim Durchblättern auf einige Unstimmigkeiten usw. gestossen: Die Haidingerschen Polarisationsbüschel (Seite 521, Teil I) sieht man natürlich nicht *nur* am blauen Himmel. Die bläulichen Garben entsprechen überdies der konventionellen Schwingungsebene und nicht der Polarisationsebene. Vom Stichwort «Föhn» (Seite 422, Teil I) wird auf «Wetterfühligkeit» verwiesen. Dieses Wort wird man aber vergeblich suchen. Die Anisotropie der Wärmeleitung in Kristallen ist, soweit ersichtlich, nirgends erwähnt worden. Die Abbildungen 4*a* und *b* beim Foucaultschen Pendelversuch (Seite 426, Teil I) sind vertauscht worden. Die Relativbewegung bei stossfreiem Loslassen des Pendels ist dementsprechend im Text nicht richtig dargestellt worden. Verwechselt wurden auch auf Seite 745 von Teil I die Zeichnungen des kubischen und des tetragonalen Koordinatensystems. Im Zusammenhang mit der Beschreibung der Auflösungsgrenze des Lichtmikroskops (Seite 56, Teil II) wird auf «Ultramikroskopie» hingewiesen. Es muss richtig heissen: «Ultraviolettmikroskopie». Unter «Ultramikroskop» (Seite 584, Teil II) wird behauptet, man könnte in Metallsolen noch Teilchen von 0,01 mμ Durchmesser (das heisst also von 0,1 Å) wahrnehmen. Die kleinsten Goldteilchen, die man sehen kann, sind etwa 100 Å gross. Die Grenze wäre also richtig mit 0,01 μ anzugeben. Strömungsdoppelbrechung sollen (Seite 493, Teil II) organische Flüssigkeiten zeigen. Die klassischen Untersuchungen von Freundlich am V_2O_5-Sol wären nach Ansicht des Referenten zu berücksichtigen gewesen. Das Material für die Polarisationsfolien heisst nicht «Heraphit» (Seite 545, Teil I), sondern «Herapathit»[1]. Im periodischen System der Elemente (Seite 777, Teil II) sind Kalium und Aluminium unrichtig bezeichnet, nämlich als «Ka» und «A». Unter den Physikern hätte J. Balmer eine Erwähnung sicher verdient.

In einem derartig umfangreichen Wörterbuch sind natürlich gewisse Unstimmigkeiten nie zu vermeiden. Die angeführten Fehler werden aber, wenigstens teilweise, nicht als belanglos anzusehen sein. Der Referent wird sich mit Recht fragen dürfen, ob nicht auch an anderen Stellen Unrichtiges zu finden sein wird. Bei einer Neuauflage wird wohl eine genaue Revision zweckdienlich sein. Vielleicht darf der Referent in diesem Zusammenhang anregen, dass unter jedem Artikel auch der Verfasser mit Anfangsbuchstaben angegeben wird.

Nach all diesen kritischen Bemerkungen soll aber betont werden, dass es sich bei diesem Buch nicht nur um ein sehr nützliches, sondern auch um ein überaus aufschlussreiches, zu einer weiteren Lektüre anregendes Nachschlagewerk handelt.

G. Boehm

[1] Nach dem Entdecker W. B. Herapath.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

Beitrag zu einem Extremalproblem über konvexe Rotationskörper

Von H. Bieri*

I. Die fundamentale Rolle der drei Masszahlen V, F, M in der Theorie der konvexen Körper, insbesondere also auch in der *Theorie der konvexen Rotationskörper*, ist des öftern beleuchtet worden[1]. Von besonderem Interesse ist das Problem, *bei zwei festgehaltenen Masszahlen die Extrema der dritten aufzusuchen und die zugehörigen Extremalkörper anzugeben.*

Infolge unvorhergesehener Schwierigkeiten[2] empfiehlt es sich, dem Problem folgende Wendung zu geben:

Vermittelst zweier der drei Koordinaten

$$x = \frac{4\pi F}{M^2}, \qquad y = \frac{48\pi^2 V}{M^3}, \qquad z = \frac{36\pi V^2}{F^3} \tag{1}$$

wird die Menge aller konvexen Rotationskörper auf eine Ebene abgebildet. *Gesucht wird der Rand des Bildes.*

Ist dieser Rand einmal bekannt, so können ohne Mühe die Eigenschaften der Extremalkörper bezüglich der Masszahlen abgelesen werden. In der vorliegenden Note wird ein bis jetzt unbekanntes Randstück aufgezeigt, und als neue Extremalkörper werden Kegel erkannt, deren Öffnungswinkel einem angebbaren abgeschlossenen Intervall $\psi_3 \leq \psi \leq \psi_4$ angehören.

II. Es ist schwierig, das Problem direkt anzupacken, bedeutet dies doch, einen Körper so zu deformieren, dass zwei Masszahlen konstant bleiben. So wurde denn indirekt vorgegangen. Nur eine Masszahl blieb fest, und die Extrema der beiden andern wurden zu bestimmen gesucht. Bei festem Äquatorradius war es überdies möglich, Ungleichungen aufzufinden, die alle drei Mass-

* Bern.

[1] H. Hadwiger, *Altes und Neues über konvexe Körper*, 3. Kapitel (Birkhäuser, Basel und Stuttgart 1955).

[2] H. Hadwiger und H. Bieri, Math. Nachr. *13*, 19 (1955).

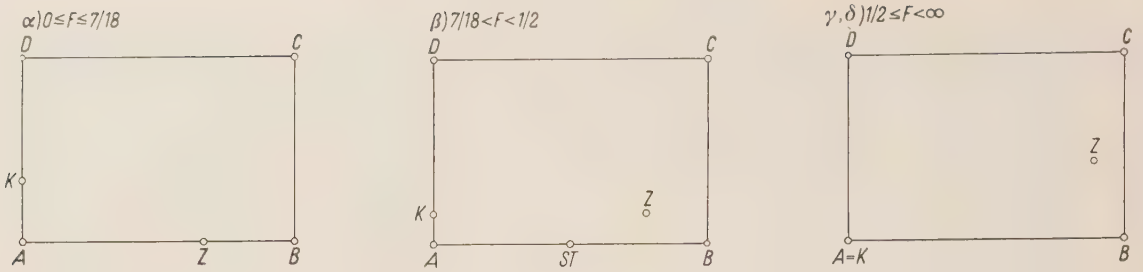

Abb. 1. – – – Kegelstumpfkurve. *K* Bildpunkt des Kegels. *Z* Bildpunkt des Zylinders. *ST* Bildpunkt des speziellen Kegelstumpfes.

zahlen enthalten[3]. Dieser Umstand ist denn auch am vorliegenden Teilerfolg massgebend beteiligt.

A. Wir betrachten zunächst die Menge aller konvexen Rotationskörper der festen Länge $l = 1$[4] und geben uns die reduzierte Oberfläche F vor, wobei $F^* = \pi F$ gesetzt werde. Es gilt:

a) α) Im Intervall $0 \leq F \leq {}^7/_{18}$ besitzen *Zylinder* und nur diese Körper kleinstes Volumen v.

β) Im Intervall ${}^7/_{18} < F < 1/2$ besitzen *spezielle Kegelstümpfe*[5] und nur sie kleinstes Volumen v. (2)

γ) Im Intervall $1/2 \leq F < \infty$ besitzen *Kegel* und nur diese Körper kleinstes Volumen v.

b) Im Intervall $0 \leq F < \infty$ besitzen *Kegel* und nur diese Körper das grösste Integral der mittlern Krümmung M[6].

c) α) Im Intervall $0 \leq F \leq 1$ sind die Extremalkörper, welche grösstes Volumen V besitzen, noch unbekannt[7]. Doch erhält man folgendermassen eine obere Schranke $\overline{V}$: Bei festem F weist der *symmetrische Doppelkegel* den *grössten Äquatorradius* auf. Der diesem Körper *umbeschriebene Zylinder* liefert $\overline{V}$.

β) Im Intervall $1 \leq F < \infty$ sind die Extremalkörper mit grösstem Volumen unter den *Nodoiden* zu suchen[8]. Nun besitzt das *Nodoid ohne Kanten* den *grössten Äquatorradius*, und der diesem Körper *umbeschriebene Zylinder* liefert V.

d) Die Extremalkörper, welche das kleinste Integral der mittlern Krümmung aufweisen, sind noch unbekannt[9]. Doch erhält man folgendermassen eine untere Schranke $\underline{M}$: Bei festem F besitzt der *Zylinder* den *kleinsten Äquatorradius*. Der diesem Körper *einbeschriebene symmetrische Doppelkegel* liefert $\underline{M}$.

Gestützt auf (1) und (2) kann man nun für festes F in einer (x,z)-Ebene *rechteckige Bereiche mit achsenparallelen Seiten* angeben, welche die Bildpunktmenge der zulässigen Körper überdecken (Abb. 1).

Unsere Bereiche sind nach oben und nach rechts sehr unscharf begrenzt. Dieser unbestreitbare Nachteil wird aufgewogen durch den Umstand, dass im dritten Bereich die Ecke links unten durch einen konvexen Rotationskörper besetzt ist. Wir haben nun die Vereinigungsmenge aller Rechteckbereiche zu studieren. Eingehende Diskussion ergibt folgendes (Abb. 2):

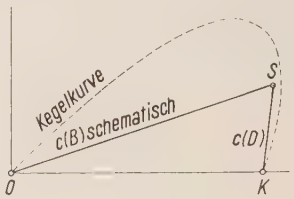

Abb. 2

α, β) Durchläuft F das Intervall $0 \leq F \leq 1/2$, so steigen *Kegel-* und *Zylinderkurve von unten links nach oben rechts an*[10]. Infolgedessen wird durch die vom Punkte B erzeugte Kurve $c(B)$, welche sich ebenso verhält, *das Bild nach unten unscharf abgegrenzt*, während die durch alle übrigen Punkte erzeugten Kurven nichts Neues liefern[11].

γ) Im Intervall $1/2 \leq F \leq F_s$ steigt die *Kegelkurve von links oben nach rechts unten ab*, und zwar ist der Anfangspunkt ein Scheitel bezüglich der x-Achse, der Endpunkt ein Scheitel bezüglich der z-Achse. *In diesem Intervall berandet also die Kegelkurve das Bild nach innen*[12].

δ) Im restlichen Intervall $F_s \leq F < \infty$ *steigt die Kegelkurve weiter ab*, und zwar *von rechts nach links*, um schliesslich im Bildpunkt der *Kreisscheibe*, K, zu münden. Bei dieser Sachlage wird nun die vom Punkte D beschriebene Kurve $c(D)$ massgebend. Sie verläuft gleich wie die Kegelkurve und liegt demnach mit Ausnahme von K *ganz rechts der Senkrechten durch K. Sie übernimmt die Rolle der innern unscharfen Bildberandung*[13].

Nun ergibt schon eine oberflächliche Prüfung, dass sich die Kurven $c(B)$ und $c(D)$ *innerhalb der Kegelkurve* schneiden (Abb. 2). *Das gewonnene Randsystem ist also leider nur unscharf*[14].

[3] H. Hadwiger, Math. Nachr. *2*, 114 (1949).

[4] Die Beschränkung von l bedeutet hier keine Beeinträchtigung der Allgemeinheit, da ja eine Aussage über die Bildpunktmenge geplant ist und die Abbildungsfunktionen (1) invariant gegenüber Ähnlichkeit sind.

[5] H. Bieri, Exper. *6*, 222 (1950).

[6] H. Bieri, Exper. *9*, 207 (1953); *12*, 369 (1956); *13*, 389 (1957).

[7] Vermutlich sind es Kappenkörper der Kugel.

[8] Vgl. hierzu die Dissertationen von G. Hormann (Göttingen 1887) und W. Howe (Berlin 1887).

[9] Einen günstigen Effekt zeigen die Verlängerungskörper der symmetrischen Kugellinsen (Zylinder + 2 kongruente Kugelkalotten) sowie die symmetrischen Kugelschichten.

[10] Vgl. hierzu: H. Bieri, Seminario Matematico de Barcelona *8*, 171 (1955). Die beiden Kurven werden dort diskutiert und auch im Bild wiedergegeben.

[11] Die Gerade $z = x$ (Kappenkörperkurve) und die Bildkurve der symmetrischen Kugelschichten sind als äussere Randstücke bekannt. Bezüglich der letztern vgl. H. Hadwiger, Portug. math. *7*, 73 (1948).

[12] In der Teilmenge der konvexen Rotationskörper mit einem F aus dem angegebenen Intervall sind also die Kegel extremal. Vgl. auch: H. Bieri, *Beiträge zum Hauptproblem der konvexen Körper*, Sitzungsberichte der Math. Vereinigung (Bern 1952).

[13] Dieser Umstand gestattet die Aussage, dass eine Teilmenge der oben angegebenen Kegel extremal bleibt im erweiterten Intervall $\pi/2 \leq F < \infty$.

[14] Alle genannten Kurven sind explizit anschreibbar und auch diskutierbar. Die genaue Berechnung lohnt sich aber nicht.

B. Bei festem Äquatorradius hat HADWIGER vollständige Resultate erhalten. Sie lassen sich unter Einführung des *Rundungsmasses* $\mu = 4\,\pi/F$ folgendermassen ausdrücken[15]:

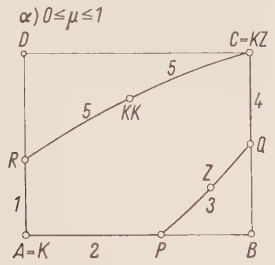

Abb. 3. K Bildpunkt des Kegels. Z Bildpunkt des Zylinders. KZ Bildpunkt des Kugelzylinders. KK Bildpunkt des Kappenkörpers der Kugel. —— Bahnkurve von P.

$$1:\ x \geq \frac{16\,\mu}{\left[\mu\left\{\pi - \arccos\left(\frac{\mu}{4-\mu}\right) + 2\sqrt{2}\,\sqrt{2-\mu}\right]^2}$$

Gleichheitszeichen für den Kegel.

$$2:\ z \geq \frac{\mu\,(2-\mu)}{2}$$

Gleichheitszeichen für den Kegel.

$$3:\ z > 9\,\mu\left[1 + \frac{\pi-2}{4}\,\mu - \frac{\sqrt{\mu}}{\sqrt{x}}\right]^2$$

Gleichheitszeichen für den Zylinder.

$$4:\ x \leq \frac{4\,\mu}{(1+\mu)^2}$$

Gleichheitszeichen für den Kugelzylinder.

$$5:\ z \leqq \mu\left[2 - \frac{\sqrt{\mu}}{\sqrt{x}}\right]^2$$

Gleichheitszeichen für Kappenkörper des Kugelzylinders.

Der wichtigste Fortschritt besteht darin, dass man die Bahnkurve des Punktes B durch diejenige des Punktes P ersetzen darf. In der Tat lässt sich zeigen, dass das Kurvenstück $\widehat{PQ}$ mit Ausnahme von P *ganz oberhalb der Bahnkurve* von P verläuft[16]. Infolgedessen wird das Bild durch die letztgenannte Kurve nach unten abgegrenzt. Die Koordinaten von P berechnet man zu

$$x = \frac{144\,\mu}{\left[12 + 3\,(\pi-2)\,\mu - 2\,\sqrt{2}\,\sqrt{2-\mu}\right]^2}; \qquad z = \frac{\mu\,(2-\mu)}{2}$$

oder mit $\mu = 2 - \nu^2$:

$$\sqrt{2} \geq \nu \geq 1$$

$$x = \frac{144\,(2-\nu^2)}{\left[6\,\pi - 2\,\sqrt{2}\cdot\nu - 3\,(\pi-2)\,\nu^2\right]^2}; \qquad z = \frac{\nu^2\,(2-\nu^2)}{2} \tag{3}$$

Beide Koordinaten wachsen monoton mit μ.

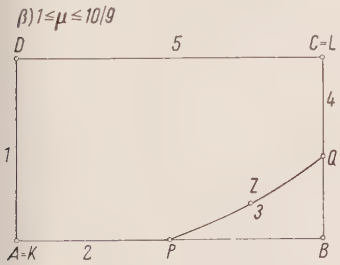

Abb. 4.
L Bildpunkt der symmetrischen Kugellinse.
1: Wie unter α).
2: Wie unter α).
3: Wie unter α).

$$4:\ x \leqq \frac{4}{\left[2\sqrt{2-\mu} + \sqrt{\mu}\cdot\arccos\sqrt{\mu(2-\mu)}\right]^2}$$

Gleichheitszeichen für die symmetrische Kugellinse.

$$5:\ z \leqq \frac{(\mu+1)^2\,(2-\mu)}{4}$$

Gleichheitszeichen für die symmetrische Kugellinse.

Wegen des Absteigens der Kegelkurve kann also auch hier, wenngleich in einem kleineren Intervall, die unter A. erwähnte Randeigenschaft der Kegelkurve festgestellt werden[17].

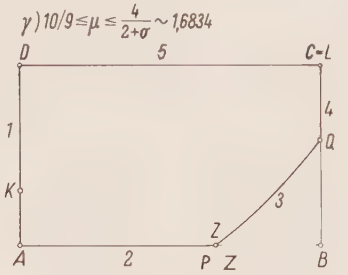

Abb. 5.
1: Wie unter α).
3: Wie unter α).
4: Wie unter β).
5: Wie unter β).

$$2:\ z > \frac{9\,\mu\,(2-\mu)^2}{16}$$

Gleichheitszeichen für den Zylinder.

[15] H. HADWIGER, Math. Nachr. *2*, 114 (1949); Math. Ann. *122*, 175 (1950); Abh. math. Sem. Hamburg. Univ. *18*, 38 (1952); Math. Nachr. *13*, 19 (1955); Math. Nachr. *14*, 377 (1956); El. Math. *12*, 101 (1957).

[16] Als Enveloppe der Bogen $\widehat{PQ}$ (Abb. 3) resultiert eine Kurve mit der Gleichung

$$x = \frac{64\,\mu}{[4+3\,(\pi-2)\mu]^2}; \qquad z = \frac{9}{64}\,\mu\,[4-(\pi-2)\mu]^2.$$

Dieselbe ist im Intervall $0 \leq \mu \leq 1$ von unten konvex, mündet in 0 mit der Steigung $^9/_{16}$ und weist ausserdem für

$$\mu = \frac{4}{3\,(\pi-2)} > 1$$

eine Spitze auf. Die Bahnkurve von P besitzt in 0 die Steigung $^4/_9 < {}^9/_{16}$, weshalb es leicht ist, zu zeigen, dass die Enveloppe im zulässigen Intervall mit Ausnahme von 0 ganz oberhalb der letztgenannten Kurve liegt. Ferner wird wegen

$$\frac{9\,\mu}{16}\,[2-(4-\pi)\,\mu]^2 < \frac{9\,\mu}{64}\,[4-(\pi-2)\,\mu]^2$$

für $0 < \mu \leq 1$ der Punkt Q vor dem Berührungspunkt mit der Enveloppe durchlaufen. Damit ist die Behauptung des Textes bewiesen.

[17] H. HADWIGER und H. BIERI, Math. Nachr. *13*, 22 (1955). Es ist $\sigma = \vartheta - 2$.

In diesem Intervall kommt neuerdings die Bahnkurve von A zur Geltung. Man beachte, dass jetzt der Bildpunkt des *Kegels* oberhalb von A liegt. Infolgedessen verläuft die Kurve $c(A)$ unterhalb der absteigenden Kegelkurve, was natürlich ungünstig ist.

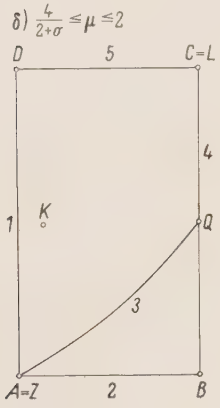

Abb. 6.
3: Wie unter α). 4: Wie unter β). 5: Wie unter β).

$$1: x \geq \frac{16\,\mu}{[(\pi-1)\,\mu+2]^2}$$

Gleichheitszeichen für den Zylinder.

$$2: z \geq \frac{9\,\mu\,(2-\mu)}{16}$$

Gleichheitszeichen für den Zylinder.

Weil die Zylinderkurve von rechts oben in K einmündet, ist diesmal die Bahnkurve von D ausschlaggebend. Ihre Koordinaten lauten:

$$x = \frac{16\,\mu}{[(\pi-1)\,\mu+2]^2}; \qquad z = \frac{(\mu+1)^2\,(2-\mu)}{4}. \tag{4}$$

Gegenüber der Variante A sind zwei Verbesserungen, leider aber auch eine Verschlechterung erzielt worden. Diese letztere hat zur Folge, dass auch diesmal kein Stück des scharfen Randes freigelegt werden kann (Abb. 7).

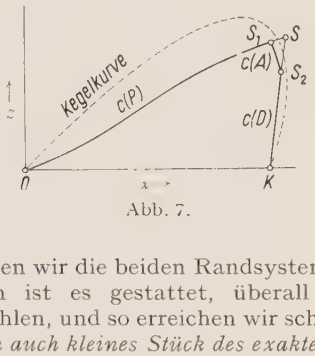

Abb. 7.

C. Nun legen wir die beiden Randsysteme zusammen. Offensichtlich ist es gestattet, überall die besseren Stücke zu wählen, und so erreichen wir schliesslich unser Ziel, *ein wenn auch kleines Stück des exakten Randes freizulegen* (Abb. 8).

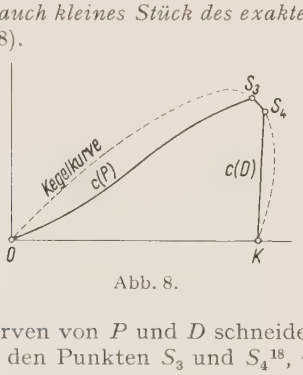

Abb. 8.

Die Bahnkurven von P und D schneiden nämlich die Kegelkurve in den Punkten S_3 und S_4[18], *und der Bogen* $\overset{\frown}{S_3 S_4}$ *der Kegelkurve berandet das Bild nach innen, und zwar scharf.*

Unter Ausnützung der *Ähnlichkeitsinvarianz* von x, z lesen wir aus den Abbildungen 8, 9 folgende Extremaleigenschaft der Kegel ab:

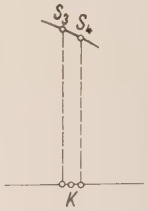

Abb. 9.

Unter allen konvexen Rotationskörpern besitzen bei vorgegebenem x gewisse Kegel[19] *im Teilintervall $x(S_3) \leq x < 8/\pi^2$ ein absolutes, im Teilintervall $8/\pi^2 \leq x \leq x\,(S_4)$ noch ein relatives Minimum von z.*

Dies ist äquivalent mit der Aussage: *Unter allen konvexen Rotationskörpern besitzen bei vorgegebener Oberfläche F und ebensolchem Integral der mittleren Krümmung M gewisse Kegel im Intervall $x(S_3) \leq x < 8/\pi^2$ ein absolutes*[20]*, im Restintervall $8/\pi^2 \leq x \leq x\,(S_4)$ noch ein relatives Minimum des Volumens V.*

Summary

The main problem of the theory of convex bodies of revolution can be stated as follows:

By means of two of the three affinely invariant functions

$$x = \frac{4\pi F}{M^2}, \qquad y = \frac{48\pi^2 V}{M^3}, \qquad z = \frac{36\pi V^2}{F^3},$$

the set of all convex bodies of revolution is mapped into a plane. The boundary of the image-set is to be determined. Part of this boundary is already known. The corresponding extremal bodies are cap-bodies of the sphere and symmetric spherical discs.

The present note is concerned with yet another, if small subset of the boundary. The corresponding extremal bodies turn out to be cones, whose angles of aperture ψ vary between well-defined limits ψ_1 and $\psi_2 > \psi_1$. F and M being given, these cones assume for $\psi_1 \leq \psi < \psi^*$ and for $\psi^* \leq \psi \leq \psi_2$ a relative and an absolute minimum, respectively.

[18] Dass die Schnittpunkte S_3, T, S_4 so liegen, wie es Abb. 8 und 9 zeigen, kann rechnerisch mit absoluter Sicherheit nachgewiesen werden unter Benützung von (3) und (4) sowie unter Ausnützung der bekannten Eigenschaften der Kegelkurve.

[19] H. Hadwiger und H. Bieri, Math. Nachr. *13*, 19 (1955). Die Sprungstelle des absoluten Minimums von V ist jetzt genau lokalisiert.

[20] Die Kegelkurve behält vermutlich ihre Randeigenschaft nach links bis zu ihrem Scheitel bezüglich der x-Achse und auch noch weiter nach rechts. Ferner wird vermutet, dass links dieses Scheitels der untere Bildrand durch die Bildkurve gewisser Kegelstümpfe und anschliessend bis 0 durch die Zylinderkurve geliefert wird. In der Umgebung von K liegen die Dinge komplizierter. Es bildet sich ein relatives Maximum des Volumens aus. Hadwiger bezeichnete als mutmassliche Extremalkörper *symmetrische Kappenkörper* von *symmetrischen Kugellinsen*. Aus dieser zweiparametrigen Schar müssten dann die richtigen Körper durch Enveloppenbildung ermittelt werden.

THEORIA

A Concept of Intracellular Transmission of Excitation by Means of the Endoplasmic Reticulum

The classical concept of cellular conduction of excitation is that an adequate change in the cell membrane environment causes an alteration of the membrane potential, which initiates a self-propagating wave of action potential and subsequent specific functional events within the cell.

Two of the major problems which arise from this concept are the following:

(1) The precise membrane, or membranes, involved. Classically the only membrane considered was the 'cell surface'.

(2) The mechanism whereby the alterations of the cell surface affect the interior of the cell.

Recent electron microscopy has differentiated the cell surface membranes which separate cytoplasm from extracellular material. It has further shown the presence of intracellular membranes (endomembranes) separating different cytoplasmic phases. Detailed references will be discussed later. From our observations of the disposition of the endomembranes, their relation to the plasma membrane, and their separation of differing cytoplasmic phases, we conclude that the intracellular membranes must possess transmembrane potentials and that they serve for the conduction of excitation as does the plasma membrane. The morphologic evidence is derived from electron micrographs of skeletal muscle fibers, smooth muscle cells, cardiac muscle cells, Purkinje conducting system cells, neurons, neuromuscular junctions, and gland cells, but it is believed that the principles apply to all cells.

The Micromorphologic Background.—In general there are either multiple or single layers of 'cell membranes' separating cells from extracellular spaces or from other cells. They comprise the plasma membranes with or without basal membrane. Within the cell are now observed 'endomembranes', separating intracellular phases. These include: (*a*) membranes of the endoplasmic reticulum, (*b*) membranes of the Golgi apparatus, (*c*) membranes of the mitochondria, and (*d*) nuclear membranes.

The different membrane systems and the several important phases separated by them are exemplified in Figure 1, which represents schematically a muscle fiber with its nerve and myoneural junction.

The sarcolemma of skeletal muscle fibers, cardiac muscle cells, and the cytolemma of smooth muscle cells consists of the outer sheath of denser ground substance (basement membrane), the inner plasma membrane, and the interspace. In addition, embryonic and adult heart muscle cells, and Purkinje conducting system cells show intercalated discs formed by adjacent plasma membranes and their interspace. The same principles apply to the nerves, in which the axonal plasma membrane is either adjacent to a basement membrane, as in vertebrate neuromuscular junctions, or to a plasma membrane, as along the sheath cell of non-myelinated nerves, along interneural synapses, and insect neuromuscular junctions.

The membranes of the endoplasmic reticulum form a continuous system of intracytoplasmic microtubules, vesicles, and clefts of extreme variability. Hence, they separate the continuous internal phase of the reticulum from the continuous cytoplasmic matrix. A second, differentiated phase is separated from the latter by the Golgi membranes, which in some cases may be continuous with the endoplasmic reticulum. The mitochondria lie discontinuously in the matrix, separated from the cytoplasmic phase by their outer membranes, and enclosing membranes that separate two intramitochondrial phases. The matrix further includes the contractile material, neurofilaments, or other specific structures dependent upon the cell type. A special situation is occupied by the nucleus. It is suspended within the fluid phase of the endoplasmic reticulum; the nucleoplasmic membrane separating the nuclear and reticulum phases. Cytoplasmic matrix and nuclear matrix, however, communicate at irregular intervals through small pore canals bounded by adjoined nuclear and reticulum membranes. The pore canal is traversed by only a fine phase border.

In muscle fibers we find an extremely regular transverse and longitudinal arrangement of the endoplasmic reticulum as emphasized in Figure 1. Its tubes are attached to the plasma membrane at the levels of the *Z* and *M* bands. The reticulum tubules surround the *Z* and *M* bands of each sarcomere, and are linked transversely and longitudinally between the fibrils. At the termini of the vertebrate myoneural junctions the reticulum tubules are attached to the digital infoldings of the muscle plasma membrane. In insects the endoplasmic reticulum tubules ramify profusely at the neuromuscular contact fields. The endoplasmic reticulum is continuous with the nuclear membrane, and closely associated with the mitochondria. In nerve cells the endoplasmic reticulum forms a structural component of the Nissl substance and extends longitudinally within the dendrites and axons.

Mitochondria of the muscle fiber are abundant immediately beneath the plasma membrane, close to the *Z* bands, and around the nucleus. They occur in large numbers in both sarcoplasm and axoplasm at the myoneural junctions. In the axon they are observed near the reticulum tubules and the axoplasmic membrane.

Membrane Systems and Potentials.—The electrical phenomena observed in cells are generally attributed to the properties of a hypothetical surface membrane. Electron micrographs reveal the plasma membrane to be the only constant component of all cell surface membranes. It is the barrier between the ion concentrations of the cytoplasmic phase and the ion concentrations of the fluid phase immediately outside. The plasma membrane has a thickness of ca. 100 Å, which is that demanded by calculations from membrane capacitance and dielectric constant. It is of considerable interest that the multiply-connecting endoplasmic reticulum is a further membrane system separating different intracellular phases and ion concentrations. Therefore, not only plasma membranes, but also the endoplasmic reticulum membranes, and other endomembranes must be considered as carrying trans-membrane potentials (see Fig. 1). Alterations of the plasma membrane potential then would induce alterations of the reticulum membrane potential by changes in cytoplasmic matrix ion concentrations at the regions of reticulum-plasma membrane juxtapositions. A potential of activation would be thus transmitted transversely and longitudinally throughout the muscle fiber. If this concept is justified, then the production of spontaneous action potentials in neurons should start in the interior, e.g., nucleus, endoplasmic reticulum (Nissl substance), and mitochondria, or by interaction of these components, to be transmitted secondarily to axon surface membranes and synapses.

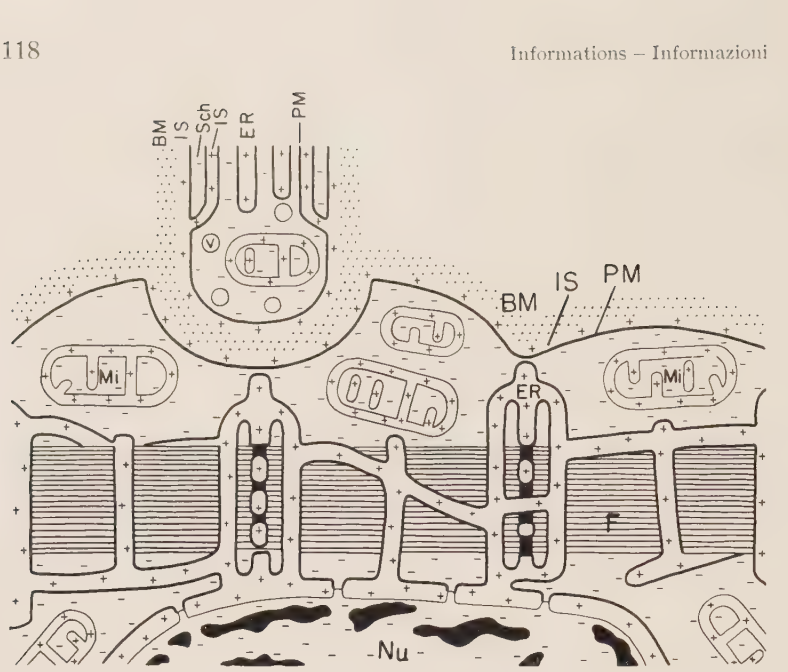

Fig. 1. — Schematic representation of vertebrate muscle fiber with myoneural junction. At upper left, nerve ending with its axonal reticulum (*ER*), vesicles (*V*), and mitochondrion (*Mi*). Axon accompanied on both sides by cytoplasm of Schwann cell (*Sch*) separated from the axon by plasma membrane interspace (*IS*). Neuron basement membrane (*BM*) (stippled) communicates with basement membrane of end plate and sarcolemma. Basement membrane (*BM*) separated from underlying plasma membranes (*PM*) by cytolemma interspace (*IS*). Mitochondria in muscle cell found beneath plasma membrane, near myoneural junction, near *Z* lines and around nucleus (*Nu*). Part of a single myofibril (*F*) is shown, with 2 sarcomeres and 2 central *Z* lines. Endoplasmic reticulum continuous throughout cell; extending transversely from plasma membrane infoldings to nuclear membrane, and longitudinally around the myofibril, with central association at *Z* and secondary at *H* regions. Known plasma membrane resting potential and inferred intracellular distribution of resting potentials indicated by plus and minus signs.

Known and Inferred Responses to Potential Alterations. The imbalance initiated by the local currents from the end plate would have the following consequences upon the entire system in the muscle fiber:

(1) Propagation of the action potential along the plasma membrane, accompanied by ion displacements.
(2) Alteration of the ion concentration surrounding the endoplasmic reticulum at its points of peripheral attachment.
(3) Propagation of a potential of activation along the membranes of the endoplasmic reticulum.
(4) Ionic imbalance around the sarcomere at the *I* (*Z*) and *H* (*M*) regions, where the reticulum is intimately associated with the fibrils.
(5) Alteration of mitochondrial trans-membrane potentials and consequent ionic imbalance in the inner mitochondrial phases.
(6) Alterations in the nuclear membrane proper, nucleoplasm, and pore regions.
(7) Energy release by inversion of membrane potentials.
(8) Restoration of ion balance and potentials.

It should be emphasized that these events are cyclic in nature and interact. For example, the mitochondria receive stimuli from the advancing cytoplasmic imbalance and are influenced by 3 carbon products from fibril activity. In turn they produce the energy for the restorative processes. As mentioned above, the events should be the reverse in neurons. Action potentials are initiated by metabolic processes in the interior and secondarily transmitted to the cell surface.

It is important to recognize that under the conditions considered nothing 'flows' or is 'transported', but that only a series of reversible local disturbances succeed each other. By the structural relationships now known a much more intimate influence of action potentials occurs upon the cellular interior than previously could be considered. Distances for ion movements are brought within the range of a few millimicrons across membranes, and the areas of influence narrowed to ½ micron or less.

Recognition of the Endoplasmic Reticulum and Theories Regarding its Function.—As a necessary background for the consideration of the function of the endoplasmic reticulum in intracellular conduction, we may note some pertinent electron microscope observations and interpretations. The presence of a discreet network of intracellular tubules and vesicles in a wide variety of cells was first shown by Porter *et al.*[1]. For more detailed information the reader is referred to recent reviews[2], and to specific papers on muscle[3], and nerve[4].

Attention was directed early to the relation of the endoplasmic reticulum (ergastoplasm) with cytoplasmic basophilia[5], and to its possible role in muscle adenylic acid metabolism[6]. Its part in nucleocytoplasmic exchange has been considered more recently, particularly in relation to exchange of RNA[7]. The proposal has been

[1] K. R. Porter, A. Claude, and E. F. Fullam, J. exp. Med. *81*, 233 (1945). – K. R. Porter, Anat. Rec. *100*, 72 (1948).

[2] K. R. Porter, J. exp. Med. *97*, 727 (1953). – W. Bernhard, A. Gautier, and C. Rouiller, Arch. Anat. micr. Morph. exp. *43*, 236 (1954).– G. E. Palade, J. biophys. biochem. Cytol. *2* (Suppl.) 85 (1956). – F. S. Sjöstrand, Int. Rev. Cytol. *5*, 456 (1956). – A. B. Novikoff, Science *124*, 969 (1956).

[3] H. S. Bennett, J. biophys. biochem. Cytol. *2* (Suppl.), 99, 16 (1956). – G. A. Edwards, H. Ruska, P. Souza Santos, and A. Vallejo-Freire, J. biophys. biochem. Cytol. *2* (Suppl.), 143 (1956). – K. R. Porter, J. biophys. biochem. Cytol. *2* (Suppl.), 163 (1956). – R. Caesar, G. A. Edwards, and H. Ruska, *Architecture and nerve supply of mammalian smooth muscle*, Ann. Rep. Div. Lab. Res., New York State Department of Health (1956) J. biophys. biochem. Cytol. *3*, 867, 1957 – H. Ruska in *Symposium on the Submicroscopic Organization and Function of Nerve Cells*, Caracas (Venezuela), Exp. Cell Res. Suppl. (in press) – K. R. Porter in *Symposium on the Submicroscopic Organization and Function of Nerve Cells*, Caracas (Venezuela), Exp. Cell Res. Suppl. (in press). – G. A. Edwards, H. Ruska, and E. de Harven, *Insect neuromuscular junctions*, J. biophys. biochem. Cytol. *4*, 107, 1958.

[4] H. Fernández-Morán, Exp. Cell Res. *3*, 5 (1952). – E. DeRobertis and F. O. Schmitt, J. cell. comp. Physiol. *31*, 1 (1948). – E. DeRobertis and H. S. Bennett, Fed. Proc. *13*, 35 (1954). – S. L. Palay and G. E. Palade, J. biophys. biochem. Cytol. *1*, 69 (1955). – B. B. Geren and F. O. Schmitt, Int. Union biol. Sci. [B] *21*, 251 (1955).– J. D. Robertson, J. biophys. biochem. Cytol. *2*, 381 (1956). – S. L. Palay, J. biophys. biochem. Cytol. *2* (Suppl.), 193 (1956).

[5] K. R. Porter, J. exp. Med. *97*, 727 (1953). – W. Bernhard, A. Gautier, and C. H. Oberling, C. R. Soc. Biol. (Paris) *145*, 566 (1951). – A. J. Dalton, H. Kahler, M. J. Striebich, and B. Lloyd, J. nat. Cancer Inst. *11*, 439 (1950). – G. E. Palade and K. R. Porter, Anat. Rec. *112*, 370 (1952).

[6] H. Ruska, Z. Naturf. *9b*, 358 (1954).

[7] K. R. Porter, J. exp. Med. *97*, 727 (1953). – W. Bernhard, A. Gautier, and C. Rouiller, Arch. Anat. micr. Morph. exp. *43*, 236 (1954). – G. E. Palade, J. biophys. biochem. Cytol. *2* (Suppl.), 85 (1956). – G. A. Edwards, H. Ruska, P. Souza Santos, and A. Vallejo-Freire, J. biophys. biochem. Cytol. *2* (Suppl.), 143 (1956). – M. L. Watson, J. biophys. biochem. Cytol. *1*, 257 (1955). – H. Ruska, Zbl. Bakt. *166*, 546 (1956).

made that exchange of inorganic ions and small molecules occurs across reticulum membranes by way of the perinuclear cisterna and that large molecules pass through the pores of the nuclear envelope[8]. Probably this mechanism represents an important circulation of gene-specific products from the nucleus to the cytoplasm and the resupply with breakdown products to be utilized once more by the nucleus[9]. By this mechanism the cytoplasm not only is regulated by, but also regulates nuclear activity. A functional parallel exists between plasma membrane (control of environment-cell exchange) and reticulum membrane (control of nucleus-cytoplasm exchange). It is important to note that the nucleo-cytoplasmic, as well as the cell surface exchange, can now be considered to be under the influence of nerve potentials.

The endoplasmic reticulum forms part of the Nissl substance[10] which loses characteristic staining properties upon exhaustion of the neuron. In like manner the endoplasmic reticulum disappears in certain liver cell injuries and during regeneration reappears first around the nucleus and immediately beneath the plasma membrane[11]. It is notable that nerve cells and cells under considerable nervous control, such as gland and muscle cells, are rich in ergastoplasm and endoplasmic reticulum respectively. A special functional situation obtains in neurosecretory cells in which nervous and secretory activity coexist, and secretory products are very likely elaborated at the expense of Nissl substance[12].

That an intracellular network exists in striated muscle, and that such a network may bear an important relation to the excitation of the contractile material was postulated in the last century[13]. On the basis of electron micrographs, Bennett and Porter[14] first suggested the possible mechanics of involvement of the reticulum during contraction. It was thought that the reticulum might itself move, or might participate in the movement of material from sarcoplasm into fibril. As a second possibility, it has been pointed out that its membranes may flow by formation in one region and breakdown at a distance; thus serving as carriers of particles and ions[15]. Two investigating groups have arrived at what we consider the most logical interpretation for the problem of conduction, i.e., that the endoplasmic reticulum possesses a trans-membrane potential and serves in impulse transmission by membrane depolarization[16].

The membranes of the endoplasmic reticulum separate phases with different protein and ion concentrations. Following fixation, the inner phase of the endoplasmic reticulum appears less opaque than the cytoplasmic matrix; indicating a lower protein concentration in this phase. The semipermeability of the membrane has been postulated[17] for the ergastoplasm of pancreas, and the suggestion made that the tubules could behave like osmometers. Experimentally it has been shown that the reticulum tubules swell osmotically in muscle degeneration following ischemia[18], and that the intrareticulum and perinuclear spaces are augmented in water resorbing intestinal epithelium[19]. These facts necessarily imply water movements, alterations of ion concentrations, a transmembrane potential, and lead to the possibility of impulse conduction.

Indeed, the endoplasmic reticulum has been considered to play a role in ion exchange, water balance, nuclear-cytoplasmic exchange, degree of shortening of the sarcomere, and conduction of excitation. The multiplicity of function derives from the morphologic arrangement and selective permeability of the reticulum membranes.

Further Evidences for the Role of Endomembranes in the Conduction of Excitation.—Davson and Danielli[20] calculated from membrane capacitance and dielectric constant a necessary thickness of the order of 100 Å for the irritable membrane (see Katz[21] for discussion of physical properties of cell membranes). Electron micrographs in fact show a plasma membrane thickness of ca. 100 Å. The same value obtains for the membranes of the endoplasmic reticulum. It has been postulated further that parts of the plasma membrane may migrate into the cell to participate in the formation of the endoplasmic reticulum[22]. A rough calculation of the surface ratio between plasma membrane and reticulum membrane, in fibrillar muscle fibers, indicates that the contact surface between reticulum and cytoplasmic matrix is at least 5 times greater than that between plasma membrane and matrix. The volume included by the reticulum membranes per surface unit compares with the space between plasma membrane and basement membrane, or between two adjacent plasma membranes in the case of the intercalated discs in heart muscle[23]. Within the membranes of the endoplasmic reticulum or between the plasma membranes of the intercalated disc, there should be little loss of ions due to the fact that these systems are closed. In the case of the sarcolemma, the basement membrane must serve as a restricting ion barrier. Otherwise more ions would be lost from the interspace to the interstitium. The lack of this restricting barrier in the intercalated disc means facilitation of longitudinal cell to cell conduction.

There is no evidence of propagated muscle potentials in insects and crustaceans[24], but probably a summing of local non-propagated potentials, suggesting that the muscle response is a series of local shortenings. Morphologic evidences of multiple nerve endings and longitudinal nerve-muscle contact in insects[25] and lobster[26]

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support the electrical data. Similar multiple nerve endings, 'small fiber system', have been shown in the frog for certain tonically reacting muscles[27] which show local, non-propagated shortening in the vicinity of the small motor nerve ending. It has been further found that the fine nerve twigs terminate opposite *Z* bands in insect muscle[28], suggesting that conduction could occur along the 'Grundmembran'. It must be noted, however, that the 'Grundmembran' concept meant the separation of two equal phases of cytoplasmic matrix and not the separation of two ionically unequal phases as achieved by the endoplasmic reticulum.

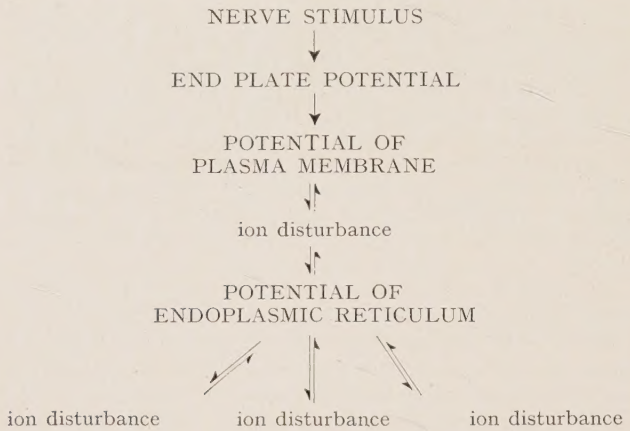

Fig. 2. – Diagram of probable sequence of events from nerve action potential through to muscle cell nucleus.

Not only the nerve-muscle relationship but also the reticulum-contractile material relationship becomes important. Phasic muscle fibers possess a well organized longitudinal and transverse endoplasmic reticulum and well defined fibrils (Fig. 1), whereas tonic fibers are characterized by non-fibrillar contractile material[29] and sparse reticulum with regular peripheral attachments but less systematic longitudinal distribution[30].

Application of small electric impulses to restricted regions of a single muscle fiber by means of a micropipette causes a response when applied to the *I* region but the same stimulus is ineffective against the *A* band. The resulting local contraction travels inwardly, indicating that the *Z* band (reticulum!) is involved in excitation and conduction[31]. Thus, it is evident that a periodic variation in thresholds and excitability exists along the muscle fiber, in correspondence with the periodic attachment of the endoplasmic reticulum to the plasma membrane at the *Z* band levels. High excitability and low threshold reside at *I* and the inverse at the *A* regions. This in turn suggests that a segmental decrement occurs in the surface action potential of muscle fibers, perhaps similar to the internodal decrement in nerves[32]. The sarcomere, together with its endoplasmic reticulum and surrounding cytoplasmic matrix, now takes the place of the muscle fiber as the functional element for potentials, tension, contraction and anaerobic metabolism. Morphologically, the sarcomere has been defined as the *Z* to *Z* distance of the contractile material. Since the contraction is symmetrical, involving the migration of the two proximal halves of the *A* region towards the *Z*, the true motor unit should be the *M* to *M* distance, with the *Z* as the center[33].

As far as can be determined from electrophoretic and other measurements[34], the cell surfaces, cytoplasmic inclusions and nuclei are positive relative to the cytoplasmic matrix, and the nuclear envelope positive to the nuclear matrix. From these determinations and the known relations of the endoplasmic reticulum, we can conceive of the charges being distributed as shown in Figure 1. It is inferred that the permeability characteristics of the endoplasmic reticulum are equal to those of the plasma membrane. The phase inside the endoplasmic reticulum differs from that outside the plasma membrane. However, both the plasma membrane and the endoplasmic reticulum membranes are exposed to the cytoplasm as a common phase. Hence, they must permit similar exchange of ions, e.g., Na and K, must possess similar mechanisms for maintenance of ionic gradients and impulse transmission, and have a common cytoplasmic source of energy for restoration. It has been shown that metabolic inhibitors have an effect on skeletal muscle different from that on nerve [35]. However, it is quite possible that several types of enzymic 'pumps' might exist according to the cytoplasmic phases and membranes of the tissues involved. The Donnan equilibrium, which applies only between homogeneous phases, does not hold for muscle and nerve cells. It has been suggested that this is due to permeability barriers or to the heterogeneity of the cell interior[36]. Such permeability barriers and heterogeneity of cell interior can now be seen as the endomembranes and the additional intracytoplasmic phases separated by them.

Taking the muscle cell as a model for the demonstration of the role of endomembranes in conduction of excitation, one can derive a general scheme of the probable sequence of potential and ionic disturbances from nerve stimulus through to muscle nucleus as presented in Figure 2.

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Zusammenfassung

Die morphologische Beobachtung von getrennten cytoplasmatischen Phasen in geschlossenen Membranen führt zur Annahme von Membranpotentialen innerhalb der Zelle. Schreibt man den Membranen des endoplasmatischen Reticulums die Eigenschaften der Plasmamembran zu, so leiten sie die Erregung ins Zellinnere und dienen als Potentialspeicher.

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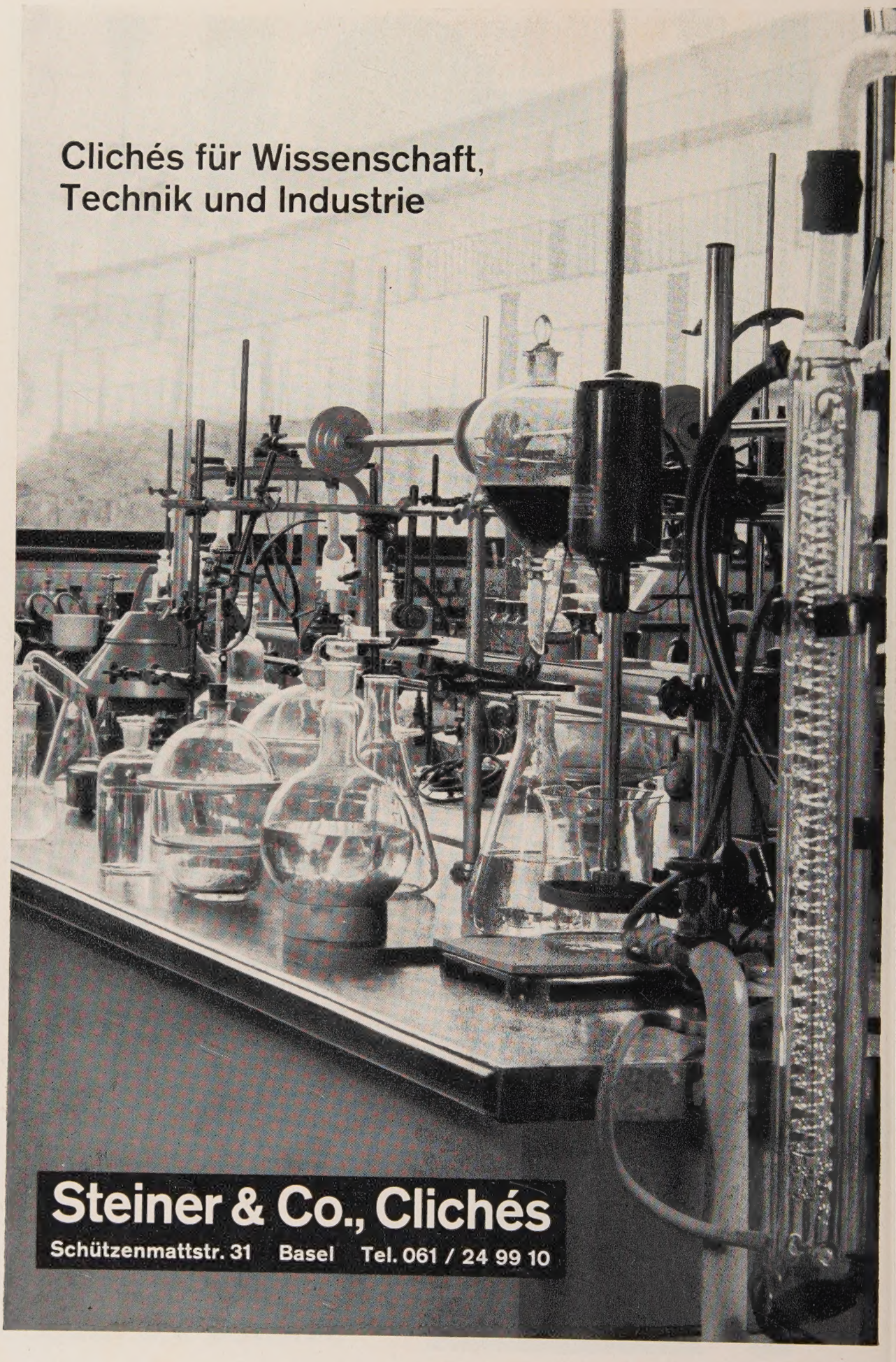
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